cobas®

Rx Only

cobas® EGFR Mutation Test v2

For in vitro diagnostic use

IVD

cobas® DNA Sample Preparation Kit	24 Tests	P/N: 05985536190
cobas® cfDNA Sample Preparation Kit	24 Tests	P/N: 07247737190
cobas® EGFR Mutation Test v2	24 Tests	P/N: 07248563190

TABLE OF CONTENTS

Intended use

Summary and explanation of the test

SECTION A: FOR USE WITH TISSUE SAMPLES

Materials and reagents

Precautions and handling requirements

Test procedure

Results

Non-clinical performance evaluation

Clinical performance evaluation

SECTION B: FOR USE WITH PLASMA SAMPLES

Materials and reagents

Precautions and handling requirements

Test procedure

Results

Non-clinical performance evaluation

Clinical performance evaluation

Additional information

Intended use

The **cobas**® EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) patients. Defined EGFR mutations are detected using DNA isolated from formalin-fixed paraffin-embedded tumor tissue (FFPET) or circulating-free tumor DNA (cfDNA) from plasma derived from EDTA anti-coagulated peripheral whole blood.

The test is indicated as a companion diagnostic to aid in selecting NSCLC patients for treatment with the targeted therapies listed in Table 1 below in accordance with the approved therapeutic product labeling:

Table 1

Drug	**FFPET**	**Plasma**
TARCEVA® (erlotinib)	Exon 19 deletions and L858R	Exon 19 deletions and L858R
TAGRISSO™ (osimertinib)	T790M	T790M*

Patients with positive **cobas**® EGFR Mutation Test v2 test results using plasma specimens for the presence of the EGFR mutations listed above are eligible for treatment with the corresponding drug as indicated in Table 1 (see Note* for T790M). Patients who are negative for these mutations by this test should be reflexed to routine biopsy and testing for EGFR mutations with the FFPET sample type.

*The efficacy of TAGRISSO™ (osimertinib) has not been established in the EGFR T790M plasma-positive, tissue-negative or unknown population and clinical data for T790M plasma-positive patients are limited; therefore testing using plasma specimens is most appropriate for consideration in patients from whom a tumor biopsy cannot be obtained.

Drug safety and efficacy have not been established for the following EGFR mutations also detected by the **cobas**® EGFR Mutation Test v2:

Table 2

Drug	**FFPET**	**Plasma**
TARCEVA® (erlotinib)	G719X, exon 20 insertions, T790M, S768I and L861Q	G719X, exon 20 insertions, T790M, S768I and L861Q
TAGRISSO™ (osimertinib)	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q

For manual sample preparation, FFPET specimens are processed using the **cobas**® DNA Sample Preparation Kit and plasma specimens are processed using the **cobas**® cfDNA Sample Preparation Kit. The **cobas z** 480 analyzer is used for automated amplification and detection.

Summary and explanation of the test

Background

Activating mutations in the gene encoding EGFR occur primarily in NSCLC, and result in constitutive activation of the kinase activity of the EGFR protein, thereby contributing to the oncogenic process.[1] The prevalence of these mutations in unselected cases of NSCLC is approximately 10% - 30%.[2, 3] However, these mutations occur more frequently, but not exclusively, in non-smoking/light-smoking female patients of Asian ancestry with adenocarcinoma histologies.[4]

The most common EGFR mutations in NSCLC include a variety of deletions in exon 19 and the substitution mutation L858R in exon 21; these mutations collectively constitute approximately 85% of EGFR mutations observed in NSCLC.[5] The **cobas**® EGFR Mutation Test v2 (**cobas**® EGFR Test) is a real-time PCR assay designed to detect G719X substitution mutations in exon 18, deletion mutations in exon 19, T790M and S768I substitution mutations in exon 20, insertion mutations in exon 20, and L858R and L861Q substitution mutations in exon 21.

The **cobas**® EGFR Test is used as a companion diagnostic test for TARCEVA®, a compound that reversibly inhibits the kinase activity of EGFR, preventing autophosphorylation of tyrosine residues associated with the receptor and thereby inhibiting further downstream signaling that promotes cell survival and proliferation. Erlotinib binding affinity for EGFR exon 19 deletion or exon 21 L858R mutations is higher than its affinity for the wild-type receptor.[6] Clinical trials have shown that patients with advanced NSCLC and with exon 19 deletion mutations or L858R substitution mutation in exon 21 that were treated with TARCEVA® as first-line treatment, are likely to experience clinical benefit compared to patients treated with chemotherapy.[3, 7]

The **cobas**® EGFR Test is used as a companion diagnostic test for TAGRISSO™ (osimertinib), an irreversible inhibitor of both EGFR TKI-sensitizing and T790M resistance mutations in advanced NSCLC. TAGRISSO™ inhibits the kinase activity of EGFR, which inhibits a cascade of intracellular downstream signaling events that promote cell proliferation, survival, and angiogenesis.[8] Clinical trials have shown that patients with advanced non-squamous NSCLC with an EGFR TKI-sensitizing mutation and have progressed following therapy with a first generation EGFR TKI and who have developed a T790M resistance mutation in exon 20 that were treated with TAGRISSO™ (osimertinib) are likely to experience clinical benefit.[9]

Table 3 lists the EGFR mutations detected by the **cobas**® EGFR Test.

Table 3 The cobas® EGFR Test is designed to detect the following mutations

Exon	EGFR Mutation Group	EGFR Nucleic Acid Sequence	COSMIC ID[10]
Exon 18	G719X	2156G>C	6239
		2155G>A	6252
		2155G>T	6253
Exon 19	Ex19Del	2240_2251del12	6210
		2239_2247del9	6218
		2238_2255del18	6220
		2235_2249del15	6223
		2236_2250del15	6225
		2239_2253del15	6254
		2239_2256del18	6255
		2237_2254del18	12367
		2240_2254del15	12369
		2240_2257del18	12370
		2239_2248TTAAGAGAAG>C	12382
		2239_2251>C	12383
		2237_2255>T	12384
		2235_2255>AAT	12385
		2237_2252>T	12386
		2239_2258>CA	12387
		2239_2256>CAA	12403
		2237_2253>TTGCT	12416
		2238_2252>GCA	12419
		2238_2248>GC	12422
		2237_2251del15	12678
		2236_2253del18	12728
		2235_2248>AATTC	13550
		2235_2252>AAT	13551
		2235_2251>AATTC	13552
		2253_2276del24	13556
		2237_2257>TCT	18427
		2238_2252del15	23571
		2233_2247del15	26038
Exon 20	S768I	2303G>T	6241
	T790M	2369C>T	6240
	Ex20Ins	2307_2308ins9GCCAGCGTG	12376
		2319_2320insCAC	12377
		2310_2311insGGT	12378
		2311_2312ins9GCGTGGACA	13428
		2309_2310AC>CCAGCGTGGAT	13558
Exon 21	L858R	2573T>G	6224
		2573_2574TG>GT	12429
	L861Q	2582T>A	6213

Principles of the procedure

The **cobas**® EGFR Test is based on two major processes: (1) manual sample preparation to obtain DNA from FFPET or plasma; and (2) PCR amplification and detection of target DNA using complementary primer pairs and oligonucleotide probes labeled with fluorescent dyes. The **cobas**® EGFR Test is designed to detect the following mutations:

- Exon 18: G719X (G719A, G719C, and G719S)
- Exon 19: deletions and complex mutations (defined as the combination of a deletion and an insertion)
- Exon 20: S768I, T790M, and insertions
- Exon 21: L858R and L861Q

Mutation detection is achieved through PCR analysis with the **cobas z** 480 analyzer. A mutant control and negative control are included in each run to confirm the validity of the run.

Sample preparation

The **cobas**® DNA Sample Preparation Kit and the **cobas**® cfDNA Sample Preparation Kit are manual sample preparations from FFPET and plasma respectively, based on nucleic acid binding to glass fibers. A protease and chaotropic lysis/binding buffer releases nucleic acids and protects the released DNA from DNases. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the DNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution. The target DNA is then amplified and detected on the **cobas z** 480 analyzer using the amplification and detection reagents provided in the **cobas**® EGFR Test kit.

PCR amplification

Target selection

The **cobas**® EGFR Test uses primers that define specific base-pair sequences for each of the targeted mutations. For the exon 19 deletion mutations, sequences ranging from 125 to 141 base pairs are targeted; for the L858R substitution mutation in exon 21, a 138 base pair sequence is targeted; for the T790M substitution mutation in exon 20, a 118 base pair sequence is targeted; for the G719X substitution mutation in exon 18, sequences ranging from 104-106 base pairs are targeted; for the S768I substitution mutation in exon 20, a 133 base pair sequence is targeted; for the exon 20 insertion mutations, sequences ranging from 125 to 143 base pairs are targeted; for the L861Q substitution mutation in exon 21, a 129 base pair sequence is targeted. Amplification occurs only in the regions of the EGFR gene between the primers; the entire EGFR gene is not amplified.

Target amplification

A derivative of *Thermus* species Z05-AS1 DNA polymerase is utilized for target amplification. First, the PCR mixture is heated to denature the DNA and expose the primer target sequences. As the mixture cools, the upstream and downstream primers anneal to the target DNA sequences. The Z05 DNA polymerase, in the presence of divalent metal cation and excess dNTP, extends each annealed primer, thus synthesizing a second DNA strand. This completes the first cycle of PCR, yielding a double-stranded DNA copy which includes the targeted base-pair regions of the EGFR gene. This process is repeated for a number of cycles, with each cycle effectively doubling the amount of amplicon DNA.

Automated real-time mutation detection

The **cobas**® EGFR Test utilizes real-time PCR technology. Each target-specific, oligonucleotide probe in the reaction is labeled with a fluorescent dye that serves as a reporter, and with a quencher molecule that absorbs (quenches) fluorescent emissions from the reporter dye within an intact probe. During each cycle of amplification, probe complementary to the single-stranded DNA sequence in the amplicon binds and is subsequently cleaved by the 5’ to 3’ nuclease activity of the

Z05-AS1 DNA Polymerase. Once the reporter dye is separated from the quencher by this nuclease activity, fluorescence of a characteristic wavelength can be measured when the reporter dye is excited by the appropriate spectrum of light. Four different reporter dyes are used to label the mutations targeted by the test. Amplification of the seven targeted EGFR sequences are detected independently across three reactions by measuring fluorescence at the four characteristic wavelengths in dedicated optical channels.

Selective amplification

Selective amplification of target nucleic acid from the sample is achieved in the **cobas**® EGFR Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP).[11] The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA but is always present in amplicon due to the use of dUTP in place of deoxythymidine triphosphate as one of the nucleotide triphosphates in the Master Mix reagents; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. The AmpErase enzyme, which is included in the Master Mix reagents, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at alkaline pH, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. The **cobas**® EGFR Test has been demonstrated to inactivate deoxyuridine-containing EGFR mutant amplicon.

**FOLLOW INSTRUCTIONS IN SECTION A FOR USE WITH TISSUE SAMPLES.
FOLLOW INSTRUCTIONS IN SECTION B FOR USE WITH PLASMA SAMPLES.**

SECTION A: FOR USE WITH TISSUE SAMPLES

Sample preparation

FFPET specimens are processed and genomic DNA isolated using the **cobas**® DNA Sample Preparation Kit, a manual specimen preparation based on nucleic acid binding to glass fibers. A deparaffinized 5-µm section of an FFPET specimen is lysed by incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released genomic DNA from DNases. Subsequently, isopropanol is added to the lysis mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the genomic DNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other cellular impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution. The amount of genomic DNA is spectrophotometrically determined and adjusted to a fixed concentration to be added to the amplification/detection mixture. The target DNA is then amplified and detected on the **cobas z** 480 analyzer using the amplification and detection reagents provided in the **cobas**® EGFR Test.

Materials and reagents

Materials and reagents provided

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® **DNA Sample Preparation Kit** 24 Tests (P/N: 05985536190)	**DNA TLB** (DNA Tissue Lysis Buffer) (P/N: 05517613001) Tris-HCl buffer Potassium chloride 0.04% EDTA 0.1% Triton X-100 0.09% Sodium azide	1 x 10 mL	   Danger H302 + H332: Harmful if swallowed or if inhaled. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H335: May cause respiratory irritation. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/eye protection/face protection. P284: Wear respiratory protection. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. P362 + P364: Take off contaminated clothing and wash before reuse.
	PK (Proteinase K) (P/N: 05860695102) Proteinase K,lyophilized	1 x 100 mg	
	DNA PBB (DNA Paraffin Binding Buffer) (P/N: 05517621001) Tris-HCl buffer 49.6% Guanidine hydrochloride 0.05% Urea 17.3% Triton X-100	1 x 10 mL	
	WB I (DNA Wash Buffer I) (P/N: 05517656001) Tris-HCl buffer 64% Guanidine hydrochloride	1 x 25 mL	
	WB II (DNA Wash Buffer II) (P/N: 05517664001) Tris-HCl buffer Sodium chloride	1 x 12.5 mL	
	DNA EB (DNA Elution Buffer) (P/N: 05517630001) Tris-HCl buffer 0.09% Sodium azide	1 x 6 mL	
	FT (Filter tubes with caps) (P/N: 05089506102)	1 x 25 pcs	
	CT (Collection Tubes) (P/N: 05880513001)	3 x 25 pcs	

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® **EGFR Mutation Test v2** 24 Tests (P/N: 07248563190)	**EGFR MMX-1** (EGFR Master Mix 1) (P/N 06471366001) Tris buffer Potassium chloride Glycerol EDTA Tween 20 3.13% Dimethyl sulfoxide 0.09% Sodium azide < 0.10% dNTPs < 0.01% Z05-AS1 DNA polymerase (microbial) <0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) < 0.01% Aptamer < 0.01% Upstream and downstream EGFR primers < 0.01% Fluorescent labeled EGFR probes	2 x 0.48 mL	N/A
	EGFR MMX-2 (EGFR Master Mix 2) (P/N 06471382001) Tris buffer Potassium chloride Glycerol EDTA Tween 20 3.13% Dimethyl sulfoxide 0.09% Sodium azide <0.10% dNTPs < 0.01% Z05-AS1 DNA polymerase (microbial) < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) < 0.01% Aptamer < 0.01% Upstream and downstream EGFR primers < 0.01% Fluorescent labeled EGFR probes	2 x 0.48 mL	N/A
	EGFR MMX-3 v2 (EGFR Master Mix 3) (P/N 07248610001) Tris buffer Potassium chloride Glycerol EDTA Tween 20 3.13% Dimethyl sulfoxide 0.09% Sodium azide < 0.10% dNTPs < 0.01% Z05-AS1 DNA polymerase (microbial) < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) < 0.01% Aptamer < 0.01% Upstream and downstream EGFR primers < 0.01% Fluorescent labeled EGFR probes	2 x 0.48 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® **EGFR Mutation Test v2** 24 Tests (P/N: 07248563190)	**MGAC** (Magnesium acetate) (P/N 05854326001) Magnesium acetate 0.09% Sodium azide	6 x 0.2 mL	N/A
	EGFR MC (EGFR Mutant Control) (P/N 06471455001) Tris buffer EDTA Poly-rA RNA (synthetic) 0.05% Sodium azide < 0.1% Plasmid DNA containing EGFR exon 18, 19, 20 and 21 sequences (microbial) < 0.1% EGFR wild-type DNA (cell culture)	6 x 0.1 mL	N/A
	DNA SD (DNA Specimen Diluent) (P/N 05854474001) Tris-HCl buffer 0.09% Sodium azide	2 x 3.5 mL	N/A

[a] Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® DNA Sample Preparation Kit*	15°C to 30°C	Once opened, stable up to 8 uses over 90 days or until the expiration date indicated, whichever comes first.
cobas® EGFR Mutation Test v2**	2°C to 8°C	Once opened, stable for 4 uses over 90 days or until the expiration date indicated, whichever comes first.

Note: With the exception of the **PK** reagent, do not freeze reagents.

* After addition of sterile, nuclease free water to **PK**, store unused reconstituted **PK** in 450 µL aliquots at -20°C. Once reconstituted, **PK** must be used within 90 days or until the expiration date, whichever comes first. After addition of absolute ethanol, store **WB I** and **WB II** at 15°C to 30°C. These working solutions are stable for 90 days or until the expiration date, whichever comes first.

** **EGFR MMX-1, EGFR MMX-2, EGFR MMX-3 v2,** and working MMX (prepared by the addition of **MGAC** to **EGFR MMX-1 or EGFR MMX-2 or EGFR MMX-3 v2**) should be protected from prolonged exposure to light. Working MMX must be stored at 2°C to 8°C in the dark. The prepared samples and controls must be added within 1 hour of preparation of the working MMX. Amplification must be started within 1 hour from the time that the processed samples and controls are added to the working MMX.

Additional materials required

Materials	P/N
Xylene (ACS, > 98.5% xylenes)	Any vendor
Absolute ethanol (200 proof, for Molecular Biology)	Sigma E7023 or Fisher Scientific BP2818-500 or equivalent
Isopropanol (ACS, > 99.5%)	Sigma 190764 or Fisher Scientific A451-1 or equivalent
Sterile, nuclease-free water (for Molecular Biology)	Any vendor
Bleach	Any vendor
70% Ethanol	Any vendor
Sterile disposable, serological 5- and 25-mL pipettes	Any vendor
cobas® 4800 System Microwell Plate (AD-plate) and sealing film	Roche 05232724001
cobas® 4800 System sealing film applicator (supplied with the installation of the **cobas**® 4800 System)	Roche 04900383001
Adjustable pipettors* (Capable of pipetting 5-1000 µL)	Any vendor
Aerosol barrier or positive displacement DNase-free tips	Any vendor
Pipet-Aid™*	Drummond 4-000-100 or equivalent
Bench top microcentrifuge* (capable of 20,000 x g)	Eppendorf 5430 or 5430R or equivalent
Two dry heat blocks capable of heating microcentrifuge tubes to 56ºC and 90ºC*	Any vendor
Locking-lid microcentrifuge tubes (1.5 mL sterile, RNase/DNase free, PCR grade)	Any vendor
Microcentrifuge tube racks	Any vendor
Spectrophotometer for measuring DNA concentration*	Any vendor
Vortex mixer*	Any vendor
Disposable gloves, powder-free	Any vendor
Calibrated thermometers for dry heat block*	Any vendor
Waterbath* capable of maintaining 37ºC	Any vendor
Single edged blade or similar	Any vendor

* All equipment should be maintained according to the manufacturer's instructions.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas z 480 Analyzer
cobas® 4800 System Control Unit with System Software version 2.1 or higher
EGFR Tissue P1 Analysis Package Software version 1.0 or higher
Barcode Reader ext USB
Printer

For more information regarding the materials sold separately, contact your local Roche representative.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay.

- For in vitro diagnostic use only.
- Safety Data Sheets (SDS) are available upon request from your local Roche office.
- This test is for use with FFPET NSCLC samples. Samples should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories[12] and in the CLSI Document M29-A4.[13]
- **DNA PBB** and **DNA TLB** contain Triton X-100, an irritant to mucous membranes. Avoid contact with eyes, skin, and mucous membranes.
- Xylene is a hazardous chemical and should be used in a chemical hood. Discard into chemical waste in accordance with local, state, and federal regulations.
- The use of sterile disposable pipettes and DNase-free pipettor tips is recommended.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling samples and kit reagents.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

Note: *Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.*

Contamination

- Gloves must be worn and must be changed between handling samples and **cobas**® EGFR Test reagents to prevent contamination. Avoid contaminating gloves when handling samples.
- Gloves must be changed frequently to reduce the potential for contamination.
- Gloves must be changed before leaving DNA Isolation areas or if contact with solutions or a sample is suspected.
- Avoid microbial and ribonuclease contamination of reagents.
- The amplification and detection work area should be thoroughly cleaned before working MMX preparation. Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas. For example, pipettors and supplies used for DNA Isolation must not be used to prepare reagents for Amplification and Detection.
- It is highly recommended that workflow in the laboratory proceed in a uni-directional manner, completing one activity before proceeding to the next activity. For example, DNA isolation should be completed before starting amplification and detection. DNA isolation should be performed in an area separate from amplification and detection. To avoid contamination of the working master mix with DNA samples, the amplification and detection work area should be thoroughly cleaned before working master mix preparation.

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents from different kits or lots.
- Do not use disposable items beyond their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **DNA TLB**, **DNA EB**, **MGAC**, **EGFR MMX-1**, **EGFR MMX-2**, **EGFR MMX-3 v2**, **EGFR MC**, and **DNA SD** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Spillage and cleaning

- **DNA PBB** and **WB I** contain guanidine hydrochloride. If liquid containing this buffer is spilled, clean with suitable laboratory detergent and water. If a spill occurs with potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the appropriate **cobas**® 4800 System - System Manual to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas z** 480 analyzer. Clean the **cobas z** 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - System Manual.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas z** 480 analyzer, consult the **cobas z** 480 analyzer Instrument Manual.

Specimen collection, transport, and storage

Note: *Handle all specimens as if they are capable of transmitting infectious agents.*

Specimen collection

NSCLC FFPET specimens have been validated for use with the **cobas**® EGFR Test.

Specimen transport, storage, and stability

NSCLC FFPET specimens can be transported at 15°C to 30°C. Transportation of FFPET specimens must comply with country, federal, state, and local regulations for the transport of etiologic agents.[14]

Stability of FFPET specimens has been verified for up to 12 months after the date of collection, when stored at 15°C to 30°C. Five micron sections mounted on slides may be stored at 15°C to 30°C for up to 60 days.

FFPET specimens are stable for either:

FFPET Specimen Type	**FFPET Block**	**5 µm FFPET Section**
FFPET Sample Storage Temperature	15°C to 30°C	15°C to 30°C
Storage Time	Up to 12 months	Up to 60 days

Processed sample storage and stability

Processed samples (extracted DNA) are stable for one of the following:

Extracted DNA Storage Temperature	**-15°C to -25°C**	**2°C to 8°C**	**15°C to 30°C**
Storage Time	Up to 3 freeze thaws over 60 days	Up to 14 days	Up to 1 day

Extracted DNA should be used within the recommended storage periods or before the expiration date of the **cobas**® DNA Sample Preparation Kit used to extract the DNA, whichever comes first.

Test procedure

Running the test

Figure 1 cobas® EGFR Test workflow with cobas® DNA Sample Preparation Kit

1	Start the system
2	Perform instrument maintenance
3	Remove samples and reagents from storage
4	Deparaffinize samples
5	Perform DNA isolation
6	Elute DNA
7	Create work order and print plate layout
8	Prepare amplification reagents
9	Load microwell plate with amplification reagents
10	Load microwell plate with sample
11	Seal microwell plate
12	Load microwell plate on the **cobas z** 480 analyzer
13	Start the run
14	Review results
15	With LIS: send results to LIS
16	Unload analyzer

Instructions for use

Note: *Only NSCLC FFPET sections of 5-micron thickness containing at least 10% tumor content by area are to be used in the* ***cobas***® *EGFR Test. Any sample containing less than 10% tumor content by area should be macro-dissected after deparaffinization.*

Note: *Refer to the* ***cobas*** *z 480 analyzer Instrument Manual for detailed operating instructions for the* ***cobas*** *z 480 analyzer.*

Note: *Dry heat blocks capable of heating locking-lid microcentrifuge tubes should be turned on and set at 56°C and 90°C.*

Run size

A single run can include from 1 to 30 samples (plus controls) per 96-well microwell plate. When running more than 24 samples, multiple **cobas**® EGFR Test kits will be required.

The **cobas**® EGFR Test contains sufficient reagents for 8 runs of 3 samples (plus controls) for a maximum of 24 samples per kit.

Reagent preparation

Prepare working reagents as shown in the table below prior to using the kit for the first time. Use a 5-mL serological pipette to dispense the water. Use 25-mL serological pipettes to dispense the ethanol. If the Proteinase K has already been reconstituted and frozen, thaw a sufficient number of aliquots to process the number of samples to be run.

Reagents	Reconstitution / Preparation
Proteinase K **(PK)**	Reconstitute Proteinase K (**PK**) by adding 4.5 mL of sterile, nuclease-free (PCR grade) water to the vial using a sterile, disposable 5-mL serological pipette. Mix by inverting the vial 5 to 10 times. Aliquot 450 µL of reconstituted **PK** into 1.5 mL locking-lid microcentrifuge tubes and store at -20°C for up to 90 days or until the expiration date, whichever comes first. If the Proteinase K has already been reconstituted and frozen, thaw sufficient number of aliquots to process the number of samples to be run prior to deparaffinization (70 µL of reconstituted **PK** is required for each sample).
Wash Buffer I **(WB I)**	Prepare working **WB I** by adding 15 mL of absolute ethanol to the bottle of **WB I**. Mix by inverting the bottle 5 to 10 times. Note on the bottle that ethanol has been added and the date. Store working **WB I** at 15°C to 30°C for up to 90 days or until the expiration date, whichever comes first.
Wash Buffer II **(WB II)**	Prepare working **WB II** by adding 50 mL of absolute ethanol to the bottle of **WB II**. Mix by inverting the bottle 5 to 10 times. Note on the bottle that ethanol has been added and the date. Store working **WB II** at 15°C to 30°C for up to 90 days or until the expiration date, whichever comes first.

All solutions stored at 15°C to 30°C should be clear. If precipitate is present in any reagent, warm the solution in a 37°C water bath until the precipitate dissolves. Do not use until all precipitate has been dissolved.

Deparaffinization of FFPET sections mounted on slides

Note: *Xylene is a hazardous chemical. All steps for deparaffinization should be performed under a chemical hood. See* ***Warnings and precautions****.*

Note: *If the sample contains less than 10% tumor content by area, the section must be macro-dissected.*

1. Add a slide with a mounted 5-micron FFPET section to a container with sufficient xylene to cover the tissue; soak for 5 minutes.
2. Transfer the slide to a container with sufficient absolute ethanol to cover the tissue; soak for 5 minutes.
3. Remove the slide from the ethanol and allow the section to air dry completely (5 to 10 minutes).
4. Perform macro-dissection if the sample contains less than 10% tumor content by area.

5. Label one 1.5 mL locking-lid microcentrifuge tube for each sample with the sample identification information.
6. Add 180 µL **DNA TLB** to the 1.5-mL locking-lid microcentrifuge tube.
7. Add 70 µL of reconstituted **PK** to the locking-lid microcentrifuge tube containing **DNA TLB**.
8. Scrape the tissue off the slide and into the locking-lid microcentrifuge tube. Immerse the tissue in the **DNA TLB/PK** mixture.
9. Continue with Step 1 of the **DNA Isolation procedure**.

Deparaffinization of FFPET sections not mounted on slides

Note: Xylene is a hazardous chemical. All steps for deparaffinization should be performed under a chemical hood. See ***Warnings and precautions.***

Note: If the sample contains less than 10% tumor content by area, the section must be mounted on a slide for macro-dissection and the procedure detailed in 'Deparaffinization of FFPET Sections Mounted on Slides' must be followed.

1. Place one 5-micron FFPET section into a 1.5 mL locking-lid microcentrifuge tube labeled with the sample identification information for each sample.
2. Add 500 µL Xylene to the locking-lid microcentrifuge tube containing the FFPET section.
3. Mix well by vortexing for 10 seconds.
4. Let the tube stand for 5 minutes at 15°C to 30°C.
5. Add 500 µL absolute ethanol and mix by vortexing for 10 seconds.
6. Let the tube stand for 5 minutes at 15°C to 30°C.
7. Centrifuge at 16,000 x g to 20,000 x g for 2 minutes. Remove the supernatant without disturbing the pellet. Discard the supernatant into chemical waste.
8. Add 1 mL absolute ethanol and vortex for 10 seconds.
9. Centrifuge at 16,000 x g to 20,000 x g for 2 minutes. Remove the supernatant without disturbing the pellet. Discard the supernatant into chemical waste.
10. If the pellet is floating in the remaining supernatant, spin again for 1 minute at 16,000 x g to 20,000 x g. Remove any remaining supernatant.
11. Dry the tissue pellet for 10 minutes at 56°C in a heating block with the tube open.
12. Make sure the ethanol is completely evaporated and the pellet is dry before proceeding to the next step.
13. If needed, dry pellets can be stored up to 24 hours at 2°C to 8°C.
14. Resuspend the tissue pellet in 180 µL **DNA TLB.**
15. Add 70 µL of reconstituted **PK.**
16. Continue with Step 1 of the **DNA isolation procedure**.

DNA isolation procedure

Note: *Process a negative control concurrently with the sample(s). Prepare the negative control by combining 180 µL **DNA TLB** and 70 µL **PK** solution in a 1.5 mL locking-lid microcentrifuge tube labeled as **NEG**. The negative control should be processed following the same procedure as the samples.*

1. Vortex the tubes containing the sample/**DNA TLB/PK** mixture and the negative control mixture (**NEG**) for 30 seconds.

Note: *The tissue must be fully immersed in the **DNA TLB/PK** mixture.*

2. Place tubes in the 56°C dry heat block and incubate for 60 minutes.
3. Vortex the tubes for 10 seconds.

Note: *The tissue must be fully immersed in the **DNA TLB/PK** mixture.*

4. Place tubes in the 90°C dry heat block and incubate for 60 minutes.

Note: *During the incubation, prepare the required number of filter tubes (FTs) with hinged caps by placing the **FT** onto a collection tube (CT) and labeling each **FT** cap with the proper sample or control identification.*

Note: *Each sample will need 1 **FT**, 3 **CTs** and 1 elution tube (1.5 mL locking-lid microcentrifuge tube).*

Note: *During the incubation, label the required number of elution tubes (1.5 mL microcentrifuge tube) with the proper sample or control identification information.*

5. Allow the tubes to cool to 15°C to 30°C. After cooling, pulse-centrifuge the tubes to collect liquid from the caps.
6. Add 200 µL **DNA PBB** to each tube; mix by pipetting up and down 3 times.
7. Incubate the tubes at 15°C to 30°C for 10 minutes.
8. Add 100 µL isopropanol to each tube; mix lysate by pipetting up and down 3 times.
9. Transfer each lysate into the appropriately labeled **FT/CT** unit.
10. Centrifuge the **FT/CT** units at 8,000 x g for 1 minute.
11. Place each **FT** onto a new **CT**. Discard the flow-through from the old **CT** into chemical waste, and properly dispose of the used **CT**.
12. Add 500 µL working **WB I** to each **FT**.

Note: *Preparation of working **WB I** is described in the **Reagent preparation** section.*

13. Centrifuge the **FT/CT** units at 8,000 x g for 1 minute.
14. Discard the flow-through in each **CT** into chemical waste. Place the **FT** back into the same **CT**.
15. Add 500 µL working **WB II** to each **FT**.

Note: *Preparation of working **WB II** is described in the **Reagent preparation** section.*

16. Centrifuge the **FT/CT** units at 8,000 x g for 1 minute.
17. Place each **FT** onto a new **CT**. Discard the flow-through from the old **CT** into chemical waste, and properly dispose of the used **CT**.
18. Centrifuge the **FT/CT** units at 16,000 to 20,000 x g for 1 minute to dry the filter membranes.
19. Place each **FT** into an elution tube (1.5 mL microcentrifuge tube) pre-labeled with sample or control identification. Discard the flow-through from the used **CT** into chemical waste, and properly dispose of the used **CT**.
20. Add 100 µL **DNA EB** to the center of each **FT** membrane without touching the **FT** membrane.
21. Incubate the **FT** with elution tube at 15°C to 30°C for 5 minutes.
22. Centrifuge the **FT** with elution tube at 8,000 x g for 1 minute to collect eluate into the elution tube. Properly dispose of the used **FT**.
23. Close the cap on the elution tube. The elution tube contains the DNA Stock. Proceed to Step 1 in the **DNA Quantitation** section.

Note: *Measurement of DNA concentration should be performed immediately after the DNA Isolation procedure and prior to storage.*

DNA quantitation

1. Mix each DNA Stock by vortexing for 5 seconds.
2. Quantify DNA using a spectrophotometer according to the manufacturer's protocol. Use **DNA EB** as the blank for the instrument. An average of two consistent readings is necessary. The two measurements should be within ±10% of each other when the DNA concentration readings are > 20.0 ng/µL. For DNA concentration readings < 20.0 ng/µL, the two measurements should be within ± 2 ng/µL. If the two measurements are not within ± 10% of each other when the DNA concentration readings are > 20.0 ng/µL or within ± 2 ng/µL when the DNA concentration readings are < 20.0 ng/µL, an additional 2 readings must be taken until the requirements are met. The average of these two new measurements should then be calculated.

*Note: The DNA Stock from the processed negative control (**NEG**) does not need to be measured.*

3. The DNA Stock concentration from the samples must be > 2 ng/µL to perform the **cobas**® EGFR Test. Three amplification/detections are run per sample, using 25 µL of a 2 ng/µL dilution of DNA Stock (total of 50 ng DNA) for each amplification/detection.

*Note: Each DNA Stock must have a minimum concentration of 2 ng/µL to perform the **cobas**® EGFR Test. If the concentration of a DNA Stock is < 2 ng/µL, repeat the deparaffinization, DNA Isolation, and DNA Quantitation procedures for that sample using two 5-µm FFPET sections. For mounted samples, after deparaffinization, combine the tissue from both sections into one tube, immerse the tissue in **DNA TLB + PK**, and perform DNA Isolation and Quantitation as described above. For unmounted samples, combine two sections into one tube and immerse the tissue in **DNA TLB + PK**, and perform DNA Isolation and Quantitation as described above. If the DNA Stock is still < 2 ng/µL, request another FFPET sample section from the referring clinical site.*

*Note: Processed samples (extracted DNA) are stable for up to 24 hours at 15°C to 30°C or up to 14 days at 2°C to 8°C or up to 60 days at -15°C to -25°C or after undergoing 3 freeze thaws when stored at -15°C to -25°C. Extracted DNA should be amplified within the recommended storage periods or before the expiration date of the **cobas**® DNA Sample Preparation Kit used to extract the DNA, whichever comes first.*

Amplification and detection

Note: To avoid contamination of working MMX with DNA samples, amplification and detection should be performed in an area separated from DNA Isolation. The amplification and detection work area should be thoroughly cleaned before working MMX preparation. For proper cleaning, all surfaces including racks and pipettors should be thoroughly wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution. Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

Instrument set-up

Refer to the **cobas z** 480 analyzer Instrument Manual for detailed instruction for the **cobas z** 480 set up.

Test order set-up

For detailed instructions on the EGFR workflow steps, refer to the **cobas**® 4800 System **cobas z** 480 analyzer Instrument Manual and Software Operator's Manual for the **cobas**® EGFR Mutation Test v2.

Generate a plate map with the position of all the samples and controls in the run. The Mutant Control is loaded into positions A01 – A03 on the plate. The Negative Control is loaded into positions B01 – B03 on the plate. Diluted samples are then added in sets of 3 columns, starting from C01 – C03 through H10 – H12, as shown in Figure 2.

Figure 2 Plate layout for the cobas® EGFR Test

Row / Column	01	02	03	04	05	06	07	08	09	10	11	12
A	MC MMX 1	MC MMX 2	MC MMX 3 v2	S7 MMX 1	S7 MMX 2	S7 MMX 3 v2	S15 MMX 1	S15 MMX 2	S15 MMX 3 v2	S23 MMX 1	S23 MMX 2	S23 MMX 3 v2
B	NEG MMX 1	NEG MMX 2	NEG MMX 3 v2	S8 MMX 1	S8 MMX 2	S8 MMX 3 v2	S16 MMX 1	S16 MMX 2	S16 MMX 3 v2	S24 MMX 1	S24 MMX 2	S24 MMX 3 v2
C	S1 MMX 1	S1 MMX 2	S1 MMX 3 v2	S9 MMX 1	S9 MMX 2	S9 MMX 3 v2	S17 MMX 1	S17 MMX 2	S17 MMX 3 v2	S25 MMX 1	S25 MMX 2	S25 MMX 3 v2
D	S2 MMX 1	S2 MMX 2	S2 MMX 3 v2	S10 MMX 1	S10 MMX 2	S10 MMX 3 v2	S18 MMX 1	S18 MMX 2	S18 MMX 3 v2	S26 MMX 1	S26 MMX 2	S26 MMX 3 v2
E	S3 MMX 1	S3 MMX 2	S3 MMX 3 v2	S11 MMX 1	S11 MMX 2	S11 MMX 3 v2	S19 MMX 1	S19 MMX 2	S19 MMX 3 v2	S27 MMX 1	S27 MMX 2	S27 MMX 3 v2
F	S4 MMX 1	S4 MMX 2	S4 MMX 3 v2	S12 MMX 1	S12 MMX 2	S12 MMX 3 v2	S20 MMX 1	S20 MMX 2	S20 MMX 3 v2	S28 MMX 1	S28 MMX 2	S28 MMX 3 v2
G	S5 MMX 1	S5 MMX 2	S5 MMX 3 v2	S13 MMX 1	S13 MMX 2	S13 MMX 3 v2	S21 MMX 1	S21 MMX 2	S21 MMX 3 v2	S29 MMX 1	S29 MMX 2	S29 MMX 3 v2
H	S6 MMX 1	S6 MMX 2	S6 MMX 3 v2	S14 MMX 1	S14 MMX 2	S14 MMX 3 v2	S22 MMX 1	S22 MMX 2	S22 MMX 3 v2	S30 MMX 1	S30 MMX 2	S30 MMX 3 v2

Where: MC= Mutant Control, NEG = Negative Control, S# = sample ID, and MMX # corresponds to Master Mix Reagent 1, 2, or 3 v2.

Note: *Any given sample must be spread across three consecutive columns in one row in order to generate a response.*

Note: *Working Master Mix 1 must be loaded into column 01, 04, 07, and 10 on the plate. Working Master Mix 2 must be loaded into column 02, 05, 08, and 11 on the plate. Working Master Mix 3 v2 must be loaded into column 03, 06, 09, and 12 on the plate.*

Note: *Up to 30 samples can be loaded onto a single plate. If more than one reagent kit is required to process all of the samples on the plate, then the kits must all be from the same lot.*

Dilution calculation of sample DNA stock

Dilution calculation for DNA stock concentrations from 2 ng/μL to 36 ng/μL

Note: *DNA stocks from samples should be diluted immediately prior to amplification and detection.*

Note: *Three amplification/detections are run for each sample requiring a total volume of 75 μL (25 μL for each of three reactions) of a 2 ng/μL dilution of DNA Stock (total of 150 ng DNA).*

1. For each sample, calculate the volume (μL) of DNA stock needed:

   μL of DNA stock = (90 μL x 2 ng/μL) ÷ DNA Stock concentration [ng/μL]

2. For each sample, calculate the volume (μL) of **DNA SD** needed:

   μL of **DNA SD** = 90 μL – μL of DNA Stock

Example:

DNA stock concentration = 6.5 ng/μL

1. μL of DNA Stock = (90 μL x 2 ng/μL) ÷ 6.5 ng/μL = 27.7 μL
2. μL of **DNA SD** = (90 μL – 27.7 μL) = 62.3 μL

Dilution calculation for DNA stock concentrations > 36 ng/μL

Note: *DNA Stocks from samples should be diluted immediately prior to amplification and detection.*

Note: *Three amplification/detections are run for each sample requiring a total volume of 75 μL (25 μL for each of three reactions) of a 2 ng/μL dilution of DNA stock (total of 150 ng DNA).*

1. At DNA Stock concentrations > 36 ng/μL, use the following formula to calculate the amount of **DNA SD** required to prepare at least 90 μL of diluted DNA stock. This is to ensure that each sample uses a minimum of 5 μL of DNA stock.
2. For each sample, calculate the volume (μL) of **DNA SD** needed to dilute 5 μL of DNA stock to 2 ng/μL:

   Vol. of **DNA SD** required in μL = [(5 μL of DNA stock x DNA stock concentration in ng/μL) / 2 ng/μL] – 5 μL

Example:

DNA stock concentration = 100 ng/μL

1. Vol. of **DNA SD** required in μL = [(5 μL x 100 ng/μL) / 2 ng/μL] – 5 μL = 245 μL
2. Use the calculated volume of **DNA SD** to dilute 5 μL of DNA stock.

Sample dilution

1. Prepare the appropriate number of 1.5 mL locking-lid microcentrifuge tubes for DNA Dilutions by labeling them with the proper sample identification.
2. Using a pipettor with an aerosol-resistant tip, pipette the calculated volumes of **DNA SD** into the respectively labeled tubes. Pipette 45 μL of **DNA SD** into a locking-lid microcentrifuge tube labeled as **NEG.**
3. Vortex each DNA stock and the negative control for 5 to 10 seconds.
4. Using a pipettor with an aerosol-resistant pipette tip (new tip for each pipetting), gently pipette the calculated volume of each DNA stock into the respective tube containing **DNA SD.** Pipette 45 μL of negative control (extracted eluate) into the **NEG** tube.
5. Cap the tubes and vortex each for 5 to 10 seconds.
6. Change gloves.

Reaction set-up

Preparation of working master mixes (MMX-1, MMX-2 and MMX-3 v2)

Note: EGFR MMX-1, EGFR MMX-2, EGFR MMX-3 v2, and working MMX are light-sensitive and must be protected from prolonged exposure to light.

Note: Due to the viscosity of the ***EGFR MMX*** *reagents and working MMX, pipette slowly to ensure all mix is completely dispensed from the tip.*

Note: The ***EGFR MMX-1, EGFR MMX-2****, and* ***EGFR MMX-3 v2*** *may appear light blue/purplish. This does not affect the performance of the reagent.*

Prepare three bulk working MMX, one containing **EGFR MMX-1**, one containing **EGFR MMX-2**, and the other containing **EGFR MMX-3 v2** in separate 1.5 mL locking-lid microcentrifuge tubes.

1. Calculate the volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2** required for each working MMX using the following formula:

   Volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2** required = (Number of Samples + 2 Controls +1) x 20 µL

2. Calculate the volume of **MGAC** required for each working MMX using the following formula:

   Volume of **MGAC** required = (Number of Samples + 2 Controls +1) x 5 µL

Use Table 4 to determine the volume of each reagent needed for the preparation of working MMX based on the number of samples included in the run.

Table 4 Volumes of reagents needed for working MMX-1, working MMX-2 and working MMX-3 v2

		# of Samples*									
		1	2	3	4	5	6	7	8	9	10
MMX	20 µL	80	100	120	140	160	180	200	220	240	260
MGAC	5 µL	20	25	30	35	40	45	50	55	60	65
Total Vol. for Each Working MMX (µL)		100	125	150	175	200	225	250	275	300	325

* Volumes for # of Samples is based on the sum of the # Samples + 2 Controls + 1

3. Remove the appropriate number of **EGFR MMX-1, EGFR MMX-2, EGFR MMX-3 v2,** and **MGAC** vials from 2°C to 8°C storage. Vortex each reagent for 5 seconds and collect liquid at the bottom of the tube before use. Label a sterile microcentrifuge tube for working MMX-1, working MMX-2, and working MMX-3 v2.
4. Add the calculated volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2** to their respective working MMX tube.
5. Add the calculated volume of **MGAC** to the working MMX tubes.
6. Vortex the tubes for 3 to 5 seconds to ensure adequate mixing.

Note: Samples and controls should be added to the microwell plate (AD-plate) within 1 hour after the preparation of the working MMXs.

Note: Use only ***cobas***® *4800 System Microwell Plate (AD-plate) and Sealing film.*

Preparation of plate

1. Pipette 25 µL of working MMX into each reaction well of the microwell plate (AD-plate) that is needed for the run. Do not allow the pipettor tip to touch the plate outside the well.
    - Add working MMX-1 (containing **EGFR MMX-1**) to the microwell plate (AD-plate) wells in columns 01, 04, 07, and 10, as needed.
    - Add working MMX-2 (containing **EGFR MMX-2**) to the microwell plate (AD-plate) wells in columns 02, 05, 08, and 11, as needed.
    - Add working MMX-3 v2 (containing **EGFR MMX-3 v2**) to the microwell plate (AD-plate) wells in columns 03, 06, 09, and 12, as needed.
2. Pipette 25 µL of **EGFR MC** into wells **A01, A02,** and **A03** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.
3. Using a new pipettor tip, pipette 25 µL of **NEG** into wells **B01, B02,** and **B03** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.

*Note: Each run must contain **EGFR MC** in wells A01, A02 and A03, and **NEG** in wells B01, B02, and B03 or the run will be invalidated by the **cobas z** 480 analyzer.*

Note: Change gloves as needed to protect against sample-to-sample contamination and external PCR reaction tube contamination.

4. Using new pipettor tips for each diluted sample DNA, add 25 µL of the first sample DNA to wells C01, C02, and C03 of the microwell plate (AD-plate), using a new tip for the addition of the sample DNA to each well; mix each well using a pipette to aspirate and dispense within the well a minimum of two times. Repeat this procedure for the DNA from each sample and follow the template in Figure 2 until all samples' DNA dilutions are loaded onto the microwell plate (AD-plate). Ensure that all liquid is collected at the bottom of the wells.
5. Cover the microwell plate (AD-plate) with sealing film (supplied with the plates). Use the sealing film applicator to seal the film firmly to the microwell plate (AD-plate).
6. Confirm that all liquid is collected at the bottom of each well before starting PCR.

Note: Amplification and detection should be started within 1 hour after the addition of the first sample DNA dilution to the working MMX.

Starting PCR

Refer to the **cobas**® EGFR Operator's Manual for detailed instructions on the EGFR workflow steps. When the "Select test" pop-up window appears, select "EGFR Tissue P1" and click the "OK" button.

Results

Interpretation of results

Note: All run and sample validation is performed by the ***cobas***® *4800 software.*

Note: A valid test run may include both valid and invalid sample results.

For a valid run, sample results are interpreted as shown in Table 5.

Table 5 Result interpretation for the cobas® EGFR Test

Test Result	Mutation Result	Interpretation
Mutation Detected	Ex19Del S768I L858R T790M L861Q G719X Ex20Ins (More than one mutation may be present)	Mutation detected in specified targeted EGFR region.
No Mutation Detected (NMD)*	N/A	Mutation not detected in targeted EGFR regions
Invalid	N/A	Sample result is invalid. Repeat the testing of samples with invalid results following the instructions outlined in the "**Retesting of samples with invalid results**" section below.
Failed	N/A	Failed run due to hardware or software failure. Contact your local Roche office for technical assistance.

* A "No Mutation Detected" result does not preclude the presence of a mutation in the targeted EGFR regions because results depend on percent mutant sequences, adequate sample integrity, absence of inhibitors, and sufficient DNA to be detected.

Retesting of samples with invalid results

1. Repeat dilution of the invalid sample DNA stock starting from "Dilution Calculation of Sample DNA Stock" and "Sample Dilution" procedures in the **Amplification and detection** section.
2. After performing the DNA stock dilution to 2 ng/µL as described in "Sample Dilution", continue with "Preparation of working master mix (**MMX-1, MMX-2** and **MMX-3 v2**)" and the remainder of the amplification and detection procedure.

Note: If the sample remains invalid after retesting or there was not enough DNA stock to prepare another dilution in Retesting of samples with invalid results, step A, repeat the entire test procedure for that sample, starting with Deparaffinization and DNA Isolation using a new 5-micron FFPET tumor section.

Quality control and validity of results

One set of **cobas**® EGFR Test Mutant Control (**EGFR MC**) (wells **A01, A02** and **A03**) and negative control (**NEG**) (wells **B01, B02** and **B03**) for working MMX-1, working MMX-2, and working MMX-3 v2 are included in each run of up to 30 samples. A run is valid if the **EGFR MC** and the **NEG** are valid. If an **EGFR MC** or **NEG** is invalid, the entire run is invalid and must be repeated. Prepare a fresh dilution of the previously isolated sample DNA Stock to set up a new microwell plate (AD-plate) with controls for amplification and detection.

Mutant control

The **EGFR MC** result must be 'Valid'. If the **EGFR MC** results are consistently invalid, contact your local Roche office for technical assistance.

Negative control

The **NEG** result must be 'Valid'. If the **NEG** results are consistently invalid, contact your local Roche office for technical assistance.

Procedural limitations

1. Test only the indicated specimen types. The **cobas**® EGFR Test has been validated for use with NSCLC FFPET tumor specimens.
2. The **cobas**® EGFR Test has only been validated using the **cobas**® DNA Sample Preparation Kit (Roche P/N: 05985536190).
3. Detection of a mutation is dependent on the number of copies present in the specimen and may be affected by sample integrity, amount of isolated DNA, and the presence of interfering substances.
4. Reliable results are dependent on adequate specimen fixation, transport, storage and processing. Follow the procedures in this Package Insert and in the **cobas**® EGFR Test Operator's Manual.
5. The effects of other potential variables such as specimen fixation variables have not been evaluated.
6. The addition of AmpErase enzyme into the **cobas**® EGFR Test Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Package Insert are necessary to avoid contamination of reagents.
7. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
8. Only the **cobas z** 480 analyzer has been validated for use with this product. No other thermal cycler with real-time optical detection can be used with this product.
9. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to another, users perform method correlation studies in their laboratory to qualify technology differences.
10. The presence of PCR inhibitors may cause false negative or invalid results.
11. Though rare, mutations within the genomic DNA regions of the EGFR gene covered by the primers or probes used in the **cobas**® EGFR Test may result in failure to detect presence of a mutation in exons 18, 19, 20, and 21 (results of "No Mutation Detected").
12. The **cobas**® EGFR Test shows cross-reactivity (results of "Mutation Detected") to the exon 19 L747S mutation, a rare acquired mutation that may confer resistance to TKI treatment.[15]
13. The **cobas**® EGFR Test is validated for use with 50 ng of DNA per reaction well. DNA input amounts lower than 50 ng per reaction well are not recommended.
14. The **cobas**® EGFR Test is a qualitative test. The test is not for quantitative measurements of percent mutation.
15. NSCLC FFPET specimens containing degraded DNA may affect the ability of the test to detect the EGFR mutations.

16. Samples with results reported as "No Mutation Detected" may harbor EGFR mutations not detected by the assay.
17. The **cobas**® EGFR Test detects EGFR mutations in NSCLC patients whose tumors have the exon 18 (G719X) substitutions, exon 19 deletions, exon 20 insertions and substitutions (T790M, S768I) and exon 21 substitutions (L858R, L861Q), but not any other EGFR mutations.

Non-clinical performance evaluation

*Note: The study descriptions below include cumulative data performed with v1 and v2 of the **cobas**® EGFR Test.*

For the non-clinical studies described below, percentage of tumor was assessed by pathology review. Bi-directional Sanger sequencing and next generation sequencing (NGS) were used to select the specimens for testing. Percentage of mutation of NSCLC FFPET specimen was determined using a NGS method.

Analytical sensitivity – limit of blank

To assess performance of the **cobas**® EGFR Test in the absence of template and to ensure that a blank sample does not generate an analytical signal that might indicate a low concentration of mutation, samples with no template and NSCLC FFPET EGFR wild-type specimens were evaluated. Using the analysis prescribed in the CLSI EP17-A2 guideline[16], the Limit of Blank was determined to be zero for all mutations.

Limit of detection using FFPET specimen blends

Three FFPET specimen DNA extracts for the exon 19 deletion mutations, four FFPET specimen DNA extracts for the L858R mutation, two dual mutant FFPET specimen DNA extracts for L858R and T790M mutations, two FFPET specimen DNA extracts for the G719A mutation, one dual mutant FFPET specimen DNA extract for T790M and G719A, one dual mutant FFPET specimen DNA extract for G719C and S768I mutation, one dual mutant FFPET specimen DNA extract for S768I and G719S, three FFPET specimen DNA extracts for the exon 20 insertion mutation, and three FFPET specimen DNA extracts for the L861Q mutation were blended with EGFR wild-type FFPET specimen extracts to achieve blends with samples targeting 10, 5.0, 2.5 and 1.25% mutation level as determined by next generation sequencing method (NGS), that was validated for the use for detecting EGFR mutations in exons 18, 19, 20, and 21. Serial dilutions of each specimen blend were prepared and eight replicates of each panel member were run using each of three **cobas**® EGFR Test kit lots (n=24/panel member). The limit of detection of each sample was determined by the lowest amount of DNA that gave an EGFR "Mutation Detected" rate of at least 95% for the targeted mutation, shown in Table 6.

Table 6 Limit of detection of the cobas® EGFR Test using FFPET specimen blends

EGFR Exon	EGFR Mutation Group	EGFR Nucleic Acid Sequence	Percent Mutation in the Panel Member to achieve ≥95% "Mutation Detected" Rate with 50 ng DNA input per reaction well (N=24 replicates)	COSMIC ID[16]
18	G719X	2156 G>C	2.5	6239
		2156 G>C	4.7	6239
		2155 G>A	3.2	6252
		2155 G>T	5.6	6253
19	Exon 19 Deletion	2235_2249del15	1.4	6223
		2236_2250del15	2.5	6225
		2239_2256del18*	4.7	6255
		2240_2254del15	7.2	12369
		2240_2257del18	13.4**	12370
		2239_2248>C	2.2	12382
		2237_2255>T*	4.1	12384
		2237_2253>TTGCT*	6.3	12416
		2238_2252del15	2.4	23571
		2238_2252del15*	5.5	23571
		2239_2257>GT*	6.0	Not Found
20	T790M	2369 C>T	2.0	6240
		2369 C>T	2.4	6240
		2369 C>T	3.0	6240
	S768I	2303 G>T	1.3	6241
		2303 G>T	2.4	6241
	Exon 20 Insertion	2307_2308insGCCAGCGTG	1.7	12376
		2319_2320insCAC	6.8	12377
		2310_2311insGGT	1.3	12378
21	L858R	2573 T>G	4.0	6224
		2573 T>G	4.2	6224
		2573 T>G	4.3	6224
		2573 T>G	4.3	6224
		2573 T>G	5.3	6224
	L861Q	2582T>A	2.1	6213
		2582T>A	2.2	6213
		2582T>A	3.4	6213

* Only a single level targeting approximately 5% mutation was tested for these non-predominant exon 19 deletion mutations present in the EURTAC cohort. Specimen DNA blends were tested across 3 study sites.

** Limit of Detection of the **cobas**® EGFR Test for this mutation is greater than 10% mutation level using the standard input of 50 ng per reaction well.

This study demonstrates that the **cobas**® EGFR Test can detect mutations in EGFR exons 18, 19, 20, and 21 with at least 5% mutation level using the standard input of 50 ng per reaction well.

Minimal tumor content

A total of 66 independent EGFR mutant specimens (i.e., 35 of exon 19 deletion mutants and 31 exon 21 L858R mutants) with tumor content ranging from 25% to 99% were tested to determine the minimum tumor content required for detecting the EGFR mutation in NSCLC specimens. None of the specimens evaluated had both an exon 19 deletion mutation and an exon 21 L858R mutation. Each specimen was tested without macro-dissection (neat), and after macro-dissection. The observed CtR values for the neat and macro-dissected slides were analyzed using Deming regression and the Bland-Altman plot (differences vs. mean). The results support the use of specimens whose tumor content is greater than 25% without macro-dissection.

An additional 10 EGFR wild-type NSCLC specimens (1-90% tumor content) and 10 EGFR mutant specimens (8-95% tumor content) were tested to determine the whether macro-dissection of low percent tumor NSCLC tumor tissue would improve detectability of the **cobas**® EGFR Test. Each specimen was tested without macro-dissection (neat), and after macro-dissection. All macro-dissected results matched all non-macro-dissected results and the expected mutation and wild-type results were observed for all 20 specimens.

In the Phase III EURTAC trial of erlotinib vs. cisplatin-based chemotherapy, NSCLC FFPET specimens with less than 10% tumor content were macro-dissected prior to EGFR mutation analysis. A subset of the EURTAC screened specimens was evaluated for EGFR mutation status by both the **cobas**® EGFR Test and the next generation sequencing (NGS) methods. Table 7 and Table 8 includes NSCLC specimens with valid paired results of EGFR exon 19 or L858R mutations combined from both the **cobas**® EGFR Test and the NGS sequencing. Using the NGS as the reference method, results showed that macro-dissection of NSCLC FFPET sections with less than 10% tumor content demonstrated comparable analytical accuracy to NSCLC FFPET section without macro-dissection.

Together, these studies support that macro-dissection is required for NSCLC FFPET sections with less than 10% tumor content prior to testing with the **cobas**® EGFR Test.

Table 7 Performance of the cobas® EGFR Test for NSCLC FFPET specimens with tumor contents ≤ 10% (macro-dissected)

Measure of Agreement	Percent Agreement (N)	95% CI
Positive Percent Agreement (PPA)	97.2% (35/36)	85.8%, 99.5%
Negative Percent Agreement (NPA)	94.5% (52/55)	85.1%, 98.1%
Overall Percent Agreement (OPA)	95.6% (87/91)	89.2%, 98.3%

Table 8 Performance of the cobas® EGFR Test for NSCLC FFPET specimens with tumor contents > 10% (not macro-dissected)

Measure of Agreement	Percent Agreement (N)	95% CI
Positive Percent Agreement (PPA)	93.0% (107/115)	86.9%, 96.4%
Negative Percent Agreement (NPA)	98.5% (199/202)	95.7%, 99.5%
Overall Percent Agreement (OPA)	96.5% (306/317)	93.9%, 98.1%

Cross-reactivity to other exon 18, 19, 20, and 21 mutations

EURTAC clinical trial specimens

The **cobas**® EGFR Test gave “Mutation Detected” results for the following EGFR mutations observed in the EURTAC clinical trial specimens (Table 9). Analytical performance of the **cobas**® EGFR Test in detecting these mutations has not been evaluated.

Table 9 Mutations observed in the EURTAC cohort determined to cross-react with the cobas® EGFR Test

Exon	Mutation Sequence	AA Change	COSMIC ID[16]
19	2236_2252>AT	E746_T751>I	26680
	2239_2253>CAA	L747_T751>Q	51527
	2234_2251>AAT	K745_T751>K	Not Found
	2236_2244del9	E746_R748>E	Not Found
	2236_2263>GAAGCAT	E746_A755>E	Not Found
	2237_2251>AAC	E746_751T>E	Not Found

AURA2 clinical trial specimens

The **cobas**® EGFR Test gave “Mutation Detected” results for the following EGFR mutations observed in the AURA2 clinical trial specimens (Table 10). Analytical performance of the **cobas**® EGFR Test in detecting these mutations has not been evaluated.

Table 10 Mutations observed in the AURA2 trial determined to cross-react with the cobas® EGFR Test

Exon	Mutation Sequence	AA Change	COSMIC ID[16]
21	2572_2573CT>AG	L858R	13553

Specificity – microorganisms and EGFR homologs

Specificity of the **cobas**® EGFR Test was evaluated by testing lung-related microorganisms, and plasmids of EGFR homologs, i.e., plasmids containing the sequences from each of the HER2, HER3, and HER4 genetic regions analogous to the sequences in EGFR exons 18, 19, 20, and 21 amplified by the **cobas**® EGFR Test.

Lung-related microorganisms

Streptococcus pneumoniae and *Haemophilus influenzae* at 4×10^5 colony forming units were found not to cross react or interfere with the **cobas**® EGFR Test when added to specimens containing wild-type and mutant EGFR sequences during the tissue lysis step.

Plasmids of EGFR homologs

Structurally related epidermal receptor tyrosine kinase protein analog sequences (EGFR/HER1, HER2, HER3 and HER4) have been shown not to cross-react with the **cobas**® EGFR Test when the potential cross-reactive sequence was added at a genomic copy number equivalent to 50 ng/PCR input to the isolated DNA stock prior to the amplification/detection procedure. A control condition without plasmid DNA was included. Results indicated that the observed mutations for all 15 tested FFPET specimens matched the expected mutation, as determined by sequencing, in the presence and absence of the added HER gene plasmid DNA. Additionally, the EGFR exon 19 mutation L747S was tested for cross reactivity. Results indicated that the **cobas**® EGFR Test cross-reacts with the EGFR exon 19 mutation L747S.

Interference

Triglycerides (37 mM, CLSI recommended high concentration[17]) and hemoglobin (2 mg/mL, CLSI recommended high concentration[17]) have been shown not to interfere with the **cobas**® EGFR Test when the potential interfering substance was added to the lysis step during the specimen preparation procedure.

Albuterol (Ventolin), Ipratropium (Atrovent), Fluticasone (Flonase), Ceftazidime (Fortaz), Imipenem-cilastin (Primaxin), Piperacillin-tazobactam, Cilastin (Cilastatin sodium), Betadine and Lidocaine were shown to not interfere with the performance of the **cobas**® EGFR Test when added to the lysis step during the specimen preparation procedure.

Necrotic tissue

NSCLC FFPET specimens with necrotic tissue content up to 60% for EGFR mutant and 85% in wild-type specimens have been shown not to interfere with the call results using the **cobas**® EGFR Test.

Repeatability

Repeatability of the **cobas**® EGFR Test was assessed using six FFPET specimens, including: two EGFR wild-type specimens; four EGFR mutant specimens, one of each: exon 19 deletion, S768I and G719X, T790M and L858R, and exon 20 insertion mutations. These specimens were tested in duplicate by two operators, using two different reagent lots and two **cobas z** 480 analyzers over four days. A total of 32 replicates were evaluated per sample. The **cobas**® EGFR Test had a correct call rate of 96.9% (186/192).

Repeatability of the **cobas**® EGFR Test was also assessed in a second study using four FFPET specimens including: one EGFR wild-type specimens; three EGFR mutant FFPET specimens, one of each: L861Q, G719X, and exon 20 insertion mutations. The specimens were tested in duplicate by two operators, using two different reagent lots and two **cobas z** 480 analyzers over multiple days. The **cobas**® EGFR Test has a correct call accuracy of 99.2% (127/128) across all specimen replicates, operators, reagent lots, and instruments combined.

Specimen handling reproducibility

The reproducibility of the **cobas**® DNA Sample Preparation Kit was examined using sections taken from three FFPET specimen blocks, one containing an exon 19 deletion mutation, one containing an L858R mutation, and one that is wild-type. Each specimen was tested in duplicate at each site on each day. The specimen sections for a given specimen were randomized and tested over a six day period across three sites using one operator at each site, one **cobas z** 480 analyzer at each site, three **cobas**® DNA Sample Preparation Kit lots, and one **cobas**® EGFR Test kit lot. On each test day, each operator isolated and tested the DNA from two NSCLC FFPET curl sections for each specimen using the **cobas**® EGFR Test. All specimens reported valid and correct results through-out the six days of testing. For all specimens and operators combined, the **cobas**® EGFR Test had a correct call rate of 100% (108/108).

Clinical performance evaluation

Clinical reproducibility study 1

An external study was performed to assess the reproducibility of the **cobas**® EGFR Test across 3 external testing sites (2 operators per site), 3 reagent lots, and 5 non-consecutive testing days, with a 13-member panel of DNA samples extracted from FFPET sections of NSCLC Wild Type (WT) and Mutant type (MT) tumor specimens. This panel included the L858R mutation in exon 21 and five different exon 19 deletion mutations. Of 92 runs, 90 (97.8%) were valid. A total of 2,340 tests were performed on the 13 panel members in 90 valid runs; all test results were valid. There were "No Mutation Detected" results in 180 valid tests of WT panel members, producing 100% agreement. Agreements were 100% for 10 of the 12 MT panel members. For panel member EX19_2240_2257del18 – 5% Mutation, agreement was 62.8% (67 of 180 test results were Mutation Not Detected). For panel member EX19_2240_2257del18 – 10% Mutation, agreement was 99.4% (1 of 180 test result was Mutation Not Detected). Results by overall agreement are presented in Table 11. The coefficient of variation (CV) was < 6% in all mutation panel members. Within each panel member, the CV was < 3.5%. For external control the overall CV was < 1.3%. The CV% was < 0.5% for between lots and < 1.2% for within-lot.

Table 11 Overall agreement estimates by panel member in the cobas® EGFR Test v2 reproducibility study 1

Panel Member	Number of Valid Tests	Agreement (N)	Agreement % (95% CI)[a]
Wild Type	180	180	100 (98.0, 100.0)
EX19_ 2235_2249del15 - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_ 2235_2249del15 - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2236_2250del15 - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2236_2250del15 - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2239_2248>C - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2239_2248>C - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2240_2254del15 - 5% Mutation	180	180	100 (98.0, 100.0)
EX19_2240_2254del15 - ≤10% Mutation	180	180	100 (98.0, 100.0)
EX19_2240_2257del18 - 5% Mutation	180	113	62.8 (55.3, 69.9)*
EX19_2240_2257del18 - ≤10% Mutation	180	179	99.4 (96.9, 100.0)*
EX21_ 2573T>G=L858R - 5% Mutation	180	180	100 (98.0, 100.0)
EX21_ 2573T>G=L858R - ≤10% Mutation	180	180	100 (98.0, 100.0)

Note: Results were in agreement when a Mutant Type panel member had a valid result of "Mutation Detected" or when Wild Type panel member had a valid result of Mutation Not Detected.

[a] 95% CI = 95% exact binomial confidence interval.

* Analytical sensitivity of the **cobas**® EGFR Test for detecting this mutation is greater than 10% mutation level using the standard input of 50 ng per reaction well.

Clinical reproducibility study 2

An external study was performed to assess the reproducibility of the **cobas**® EGFR Test across 3 testing sites (2 external and 1 internal, 2 operators per site), 3 reagent lots, and 5 non-consecutive testing days, with an 11-member panel of DNA samples extracted from FFPET sections of NSCLC Wild Type (WT) and mutant tumor specimens. This panel included the exon 18 G719X mutation, exon 20 T790M mutation, exon 20 S768I mutation, exon 20 insertion mutation, and exon 21 L861Q mutation. Of 91 runs, 90 (98.9%) were valid. A total of 1,980 tests were performed with 11 panel members tested in duplicate in 90 valid runs; all test results were valid. There were no Mutation Detected results in 180 valid tests of WT panel members, producing 100% agreement. Agreements were 100% for all mutant panel members, except for the Exon 20 Insertion LOD panel member. Results by overall agreement are presented in Table 12 below. The coefficient of variation (CV) was < 9.2% in all mutant panel members. For the external control, the overall CV was ≤ 1.3%. The CV was ≤ 0.6% between lots and ≤ 1.1% within-lot.

Table 12 Overall agreement estimates by panel member in the cobas® EGFR Test reproducibility study 2

Panel Member	Number of Valid Tests	Agreement (N)	Agreement % (95% CI)*
Wild Type	180	180	100 (98.0, 100.0)
Exon 18 G719X - LOD	180	180	100 (98.0, 100.0)
Exon 20 T790M - LOD	180	180	100 (98.0, 100.0)
Exon 20 S768I - LOD	180	180	100 (98.0, 100.0)
Exon 20 Insertion - LOD	180	166	92.2 (87.3, 95.7)
Exon 21 L861Q - LOD	180	180	100 (98.0, 100.0)
Exon 18 G719X - 2X LOD	180	180	100 (98.0, 100.0)
Exon 20 T790M - 2X LOD	180	180	100 (98.0, 100.0)
Exon 20 S768I - 2X LOD	180	180	100 (98.0, 100.0)
Exon 20 Insertion - 2X LOD	180	180	100 (98.0, 100.0)
Exon 21 L861Q - 2X LOD	180	180	100 (98.0, 100.0)

Note: Results were in agreement when a Mutant Type panel member had a valid result of mutation detected for the target mutation or when a Wild Type panel member had a valid result of NMD.

* 95% CI = 95% exact binomial confidence interval.

CI = confidence interval; LOD = limit of detection; NMD = No Mutation Detected

Correlation to reference method using Phase III samples from EURTAC trial

The clinical performance of the **cobas**® EGFR Test was assessed by comparing it to two reference methods – 2x bidirectional Sanger sequencing and quantitative next generation sequencing (NGS) – using 487 formalin-fixed paraffin-embedded lung tumor specimens from patients with advanced NSCLC who were screened in the Phase III EURTAC trial of erlotinib vs. cisplatin-based chemotherapy.[6,18] The clinical and demographic characteristics of the patients whose specimens were available for this retrospective testing were comparable to those of otherwise eligible patients (557) whose specimens were not available for retesting.

A total of 1,276 patients were screened for the EURTAC trial using a combination of laboratory developed tests, collectively referred to as the clinical trial assay (CTA). After excluding ineligible patients and those without CTA results, 1,044 patients were potentially eligible for the current study. Among the 1,044 eligible patients, 225 patients had samples that were mutation positive by CTA, 792 had samples that were Wild Type by CTA, and 27 had samples with inconclusive results by CTA. Of the 1,044 potentially eligible patients, 487 specimens were available for retesting with the **cobas**® EGFR Test.

All 487 specimens were tested in a blinded fashion with both the **cobas**® EGFR Test and Sanger sequencing. Of those, 406 had valid results by both the **cobas**® EGFR Test and Sanger sequencing, 38 invalid results were observed by the **cobas**® EGFR Test and Sanger sequencing, 38 invalid results by Sanger sequencing only, and 5 invalid results by the **cobas**® EGFR Test only. Among the 487 specimens available for retesting with the **cobas**® EGFR Test, 444 specimens gave valid **cobas**® EGFR Test results and were also tested with NGS. Of those, there were 36 invalid results by NGS; thus, 408 had valid results by both the **cobas**® EGFR Test and NGS. The analytical accuracy of the **cobas**® EGFR Test compared with each reference method was evaluated by estimating the positive percentage agreement (PPA), negative percentage agreement (NPA), and overall percentage agreement (OPA) and their corresponding 95% CIs for exon 19 deletions and L858R mutations in aggregate, and separately.

In the EURTAC cohort, the **cobas**® EGFR Test detected mutations in exon 19 and exon 21 of the EGFR gene as listed in Table 13. Of the mutations detected in the EURTAC cohort, analytical sensitivity was demonstrated on the mutations listed in Table 6.

Table 13 Mutations detected by the cobas® EGFR Test in the EURTAC cohort

Exon	Mutation Sequence	AA Change	COSMIC ID[15]
19	2235_2249del15	E746_A750delELREA	6223
	2236_2250del15	E746_A750delELREA	6225
	2239_2256del18	L747_S752delLREATS	6255
	2240_2254del15	L747_T751delLREAT	12369
	2240_2257del18	L747_P753>S	12370
	2239_2248>C	L747_A750>P	12382
	2239_2251>C	L747_T751>P	12383
	2237_2255>T	E746_S752>V	12384
	2237_2253>TTGCT	E746_T751>VA	12416
	2237_2257>TCT	E746_P753>VS	18427
	2238_2252del15	L747_T751delLREAT	23571
	2236_2252>AT	E746_T751>I	26680
	2239_2253>CAA	L747_T751>Q	51527
	2234_2251>AAT	K745_T751>K	Not Found
	2236_2244del9	E746_R748>E	Not Found
	2236_2263>GAAGCAT	E746_A755>E	Not Found
	2237_2251>AAC	E746_751T>E	Not Found
	2239_2257>GT	L747_P753>V	Not Found
21	2573 T>G	L858R	6224

A total of 406 samples with valid **cobas**® EGFR Test and Sanger results were included in the agreement analysis. The PPA between the **cobas**® EGFR Test and Sanger sequencing was 96.6% (95% CI: 91.5% to 98.7%), and the NPA was 88.3% (95% CI: 84.1% to 91.5%), in the detection of exon 19 deletions and L858R mutations in aggregate as presented in Table 14. The OPA was 90.6%, with the lower limit of the 95% CI above 87%. The PPA, NPA, and OPA in the detection of exon 19 deletion mutations were all > 92%. The PPA, NPA, and OPA in the detection of L858R mutations compared were all > 95%.

Table 14 Comparison of the cobas® EGFR Test with Sanger sequencing for the detection of EGFR exon 19 deletion mutations and L858R mutation

Mutation	Measure of Agreement	Percent Agreement (N)	95% CI
Exon 19 Deletion	Positive Percent Agreement (PPA)	97.3% (71/73)	90.5%, 99.2%
	Negative Percent Agreement (NPA)	92.5% (308/333)	89.2%, 94.9%
	Overall Percent Agreement (OPA)	93.3% (379/406)	90.5%, 95.4%
L858R	Positive Percent Agreement (PPA)	95.3% (41/43)	84.5%, 98.7%
	Negative Percent Agreement (NPA)	97.5% (354/363)	95.4%, 98.7%
	Overall Percent Agreement (OPA)	97.3% (395/406)	95.2%, 98.5%
Aggregate	Positive Percent Agreement (PPA)	96.6% (112/116)	91.5%, 98.7%
	Negative Percent Agreement (NPA)	88.3% (256/290)	84.1%, 91.5%
	Overall Percent Agreement (OPA)	90.6% (368/406)	87.4%, 93.1%

A total of 408 samples with valid **cobas**® EGFR Test and NGS results were included in the agreement analysis. By comparison, the PPA and NPA between the **cobas**® EGFR Test and NGS for the detection of exon 19 deletions and the L858R point mutation in aggregate were 94.0% (95% CI: 89.1% to 96.8%) and 97.7% (95% CI: 95.0% to 98.9%), respectively as presented in Table 15. The OPA was 96.3%, with a lower limit of the 95% CI of 94.0%. The PPA, NPA, and OPA in detecting exon 19 deletion mutations were all >95%, with all the 95% lower limit CIs > 90%. The PPA, NPA, and OPA in detecting the L858R mutation were also all >95%, with all lower limits of the 95% CIs ≥ 95% except for PPA (90%), due to the small number of L858R mutations detected.

Table 15 Comparison of the cobas® EGFR Test with NGS for the detection of EGFR exon 19 deletion mutations and L858R mutation in aggregate

Mutation	Measure of Agreement	Percent Agreement (N)	95% CI
Exon 19 Deletion	Positive Percent Agreement (PPA)	95.9% (94/98)	90.0%, 98.4%
	Negative Percent Agreement (NPA)	99.7% (309/310)	98.2%, 99.9%
	Overall Percent Agreement (OPA)	98.8% (403/408)	97.2%, 99.5%
L858R	Positive Percent Agreement (PPA)	90.6% (48/53)	79.7%, 95.9%
	Negative Percent Agreement (NPA)	98.6% (350/355)	96.7%, 99.4%
	Overall Percent Agreement (OPA)	97.5% (398/408)	95.5%, 98.7%
Aggregate	Positive Percent Agreement (PPA)	94.0% (142/151)	89.1%, 96.8%
	Negative Percent Agreement (NPA)	97.7% (251/257)	95.0%, 98.9%
	Overall Percent Agreement (OPA)	96.3% (393/408)	94.0%, 97.8%

Correlation to reference method using Phase II samples from AURA2

The clinical performance of the **cobas**® EGFR Test was assessed by comparing it with a validated next generation sequencing (NGS) platform using 383 formalin-fixed paraffin-embedded lung tumor specimens from patients with advanced NSCLC who were screened using the **cobas**® EGFR Mutation Test in the Phase II AURA2 trial of TAGRISSO™.

A total of 472 patients were screened for the AURA2 trial using the **cobas**® EGFR Test. After excluding ineligible patients, 383 patients were potentially eligible for the current study.

All 383 specimens were tested in a blinded fashion with both the **cobas**® EGFR Test and a validated NGS method. Of those, 368 had valid results by both the **cobas**® EGFR Test and NGS. A total of 2 invalid results were observed by both the **cobas**® EGFR Test and NGS, 2 invalid results by NGS only, and 11 invalid results by the **cobas**® EGFR Test only. The analytical accuracy of the **cobas**® EGFR Test compared with the reference method, NGS, for detection of the T790M mutation was evaluated by estimating the positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA), and their corresponding 95% CIs for the T790M mutation.

In the AURA2 trial, the **cobas**® EGFR Test detected the mutations of the EGFR gene as listed in Table 16. Of the mutations detected in the AURA 2 trial, analytical sensitivity was demonstrated on the mutations listed in Table 6.

Table 16 Mutations detected by the cobas® EGFR Test in the AURA 2 cohort

Exon	Mutation Sequence	AA Change	COSMIC ID[15]
18	2156G>C	G719A	6239
	2155G>A	G719S	6252
	2155G>T	G719C	6253
19	2239_2247delTTAAGAGAA	L747_E749delLRE	6218
	2235_2249del15	E746_A750delELREA	6223
	2236_2250del15	E746_A750delELREA	6225
	2239_2256del18	L747_S752delLREATS	6255
	2240_2254del15	L747_T751delLREAT	12369
	2240_2257del18	L747_P753>S	12370
	2239_2248>C	L747_A750>P	12382
	2239_2251>C	L747_T751>P	12383
	2237_2255>T	E746_S752>V	12384
	2237_2251del15	E746_T751>A	12678
	2235_2248>AATTC	E746_A750>IP	13550
	2235_2252>AAT	E746_T751>I	13551
	2253_2276del24	S752_I759delSPKANKEI	13556
	2237_2257>TCT	E746_P753>VS	18427
20	2369C>T	T790M	6240
	2303G>T	S768I	6241
21	2573T>G	L858R	6224
	2573_2574TG>GT	L858R	12429
	2582T>A	L861Q	6213

A total of 368 samples with valid **cobas**® EGFR Test and NGS results were included in the agreement analysis. The PPA between the **cobas**® EGFR Test and NGS was 88.3% (95% CI: 83.8% to 91.7%), the NPA was 97.3% (95% CI: 92.4% to 99.1%), and the OPA was 91.0% (95% CI: 87.7% to 93.5%) for the detection of the T790M mutation as presented in Table 17. Thirty samples were positive by NGS but negative by the **cobas**® EGFR Test: in 11/30 samples, the percent T790M mutation determined by NGS was below LoD (< 2% mutation) of the **cobas**® EGFR Test. In 19/30 samples, a moderately delayed IC Ct value indicate poor amplifiability of the DNA template.

Table 17 Comparison of the cobas® EGFR Test with NGS for the detection of the EGFR T790M mutation

Measure of Agreement	Percent Agreement (N)	95% CI
Positive Percent Agreement (PPA)	88.3% (226/256)	83.8%, 91.7%
Negative Percent Agreement (NPA)	97.3% (109/112)	92.4%, 99.1%
Overall Percent Agreement (OPA)	91.0% (335/368)	87.7%, 93.5%

Clinical outcome data

EURTAC

The EURTAC trial[18] was a Phase III, multicenter, open-label, randomized study of TARCEVA® (erlotinib) versus standard platinum doublet chemotherapy as first-line therapy in chemotherapy-naïve patients with advanced NSCLC whose tumors harbored EGFR exon 19 deletions or exon 21 (L858R) substitution mutations, as assessed by a clinical trial assay (CTA). The study was conducted under the sponsorship of the Spanish Lung Cancer Group (SLCG). A total number of 174 patients were enrolled into the study. The trial results showed that patients who received TARCEVA® had a statistically significant increase in progression-free survival (PFS) (median PFS 10.4 months vs. 5.1 months) as compared to patients who received chemotherapy, with a hazard ratio of 0.34 ($p < 0.0001$, 95% CI [0.21; 0.54]). The response rate of patients on the TARCEVA® arm was greater than the response rate of patients treated with chemotherapy (65.1% vs. 16.1%). No significant difference was observed in overall survival (OS) in the two arms, as 76% of patients on the standard chemotherapy arm crossed over to receive TARCEVA®.

Of the 174 patients enrolled into the EURTAC trial, 134 cases (77% of the study population, including 69 patients from the erlotinib arm and 65 patients from the chemotherapy arm) were available for retesting and tested retrospectively by the **cobas**® EGFR Test. Of the 134 **cobas**® EGFR Test retested cases, 116 cases (59 patients from the erlotinib arm and 57 patients from the chemotherapy arm) were "Mutation Detected" by the **cobas**® EGFR Test. Analysis of the 116 subset revealed that those patients treated with TARCEVA® had a significant increase in PFS time (median PFS 10.4 vs. 5.4 months and less likely to have an event of progressive disease or death (HR= 0.34, 95% CI [0.21;0.54], $p < 0.0001$) than patients treated with chemotherapy (Figure 3). The response rate in the TARCEVA® arm was greater compared to the chemotherapy arm (59.3% vs. 14.0%). No significant difference in OS was observed between the two groups. The observed clinical benefit in the subset of patients tested with the **cobas**® EGFR Test was comparable to that observed in the full study population (Table 18).

Additional efficacy analysis was conducted to consider patients who were tested positive by the **cobas**® EGFR Test but were tested negative or invalid by the CTA. In the worst case scenario (assuming a hazard ratio of 1 for patients positive by the **cobas**® EGFR Test and negative by CTA), data demonstrated a hazard ratio of 0.42 (95% CI [0.26; 0.57]).

Figure 3 **Kaplan-Meier plot of PFS by treatment for patients with mutation detected by the cobas® EGFR Test (investigator assessment)**

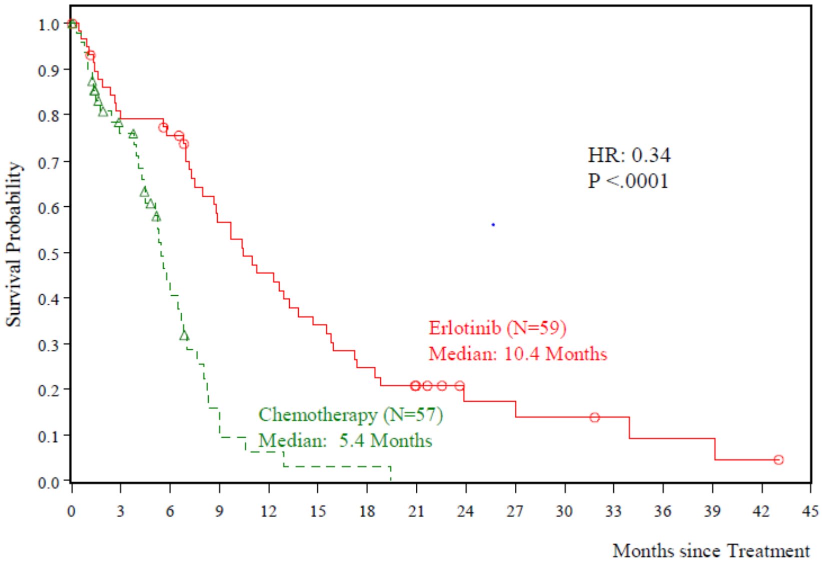


Table 18 **Clinical benefit of patients tested with the cobas® EGFR Test is comparable to that observed in the EURTAC population**

Parameter	**cobas® positive Population n = 116**		**EURTAC n = 173***	
	Chemotherapy n = 57	**Erlotinib n = 59**	**Chemotherapy n = 87**	**Erlotinib n = 86**
PFS				
Median (Months)	5.4	10.4	5.1	10.4
Hazard Ratio	0.34		0.34	
Hazard Ratio 95% CI	[0.21; 0.54]		[0.23; 0.49]	
P-Value (log-rank test)	<0.0001		<0.0001	

* One patient withdrew consent after completion of the EURTAC study, which resulted in a dataset of n = 173

AURA2

The AURA2 trial was a Phase II, multicenter, open-label, single-arm study, assessing the safety and efficacy of TAGRISSO™ as a second or ≥ third-line therapy in patients with advanced NSCLC, who had progressed following prior therapy with an approved EGFR TKI agent. All patients were required to have T790M mutation-positive NSCLC as detected by the **cobas**® EGFR Test. The primary efficacy outcome measure was objective response rate (ORR) according to RECIST 1.1 as evaluated by Blinded Independent Central Review (BICR) using the evaluable for response analysis set. The ORR was defined as the number (%) of patients with at least 1 visit response of complete response (CR) or partial response (PR) that was confirmed at least 4 weeks later (i.e., a best objective response [BOR] of CR or PR).

Of the 472 patients screened for the AURA2 trial, 383 patients were eligible for testing with the **cobas**® EGFR Test. Of those eligible, 233 T790M+ patients were recruited into the AURA2 trial, and 210 patients were enrolled and received TAGRISSO™ (full analysis set [FAS]).

Table 19 below presents the ORR by BICR and investigator assessment in AURA2. Of 210 patients who received at least one dose of TAGRISSO™ (FAS), 128 were confirmed responders by BICR with an ORR of 61.0% (95% CI: 54.0% to 67.6%) and 135 by investigator assessment with an ORR of 64.3% (95% CI: 57.4% to 70.8%).

All 383 patients eligible for AURA2 trial, were retested by the **cobas**® EGFR Mutation Test v2. Of 233 T790M positive patients recruited into the AURA 2 trial, 225 were T790M+ by the **cobas**® EGFR Mutation Test v2 and 204 were in the FAS.

Of 204 patients who received TAGRISSO™ (FAS), 127 were confirmed responders by BICR with an ORR of 62.3% (95% CI: 55.2% to 68.9%) and 133 by investigator assessment with an ORR of 65.2% (95% CI: 58.2% to 71.7%).

Table 19 Clinical benefit of T790M+ patients tested with the cobas® EGFR Test in the AURA2 trial

		AURA2			**cobas® EGFR Test (IVD) T790M Positive**		
Analysis Set	**Assessed by**	**N**	**Number of Confirmed Responders**	**ORR (95% CI)**	**N**	**Number of Confirmed Responders**	**ORR (95% CI)**
Full Analysis Set (FAS)	Blinded Independent Central Review	210	128	61.0% (54.0%, 67.6%)	204	127	62.3% (55.2%, 68.9%)
	Investigator		135	64.3% (57.4%,70.8%)		133	65.2% (58.2%, 71.7%)

SECTION B: FOR USE WITH PLASMA SAMPLES

Sample preparation

Plasma samples are processed and circulating cell free DNA (cfDNA) isolated using the **cobas®** cfDNA Sample Preparation Kit, a generic manual sample preparation based on nucleic acid binding to glass fibers. Two milliliters (mL) of plasma are processed with a protease and chaotropic binding buffer that protects the cfDNA from DNases. Subsequently, isopropanol is added to the binding mixture that is then centrifuged through a column with a glass fiber filter insert. During centrifugation, the cfDNA is bound to the surface of the glass fiber filter. Unbound substances, such as salts, proteins and other impurities, are removed by centrifugation. The adsorbed nucleic acids are washed and then eluted with an aqueous solution. The target DNA is then amplified and detected on the **cobas z** 480 analyzer using the amplification and detection reagents provided in the **cobas®** EGFR Test kit.

Materials and reagents

Materials and reagents provided

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® cfDNA Sample Preparation Kit 24 Tests (P/N: 07247737190)	**PK** (Proteinase K) (P/N: 05860695102) Proteinase K, lyophilized	2 x 100 mg	   Danger H302 + H332: Harmful if swallowed or if inhaled. H315: Causes skin irritation. H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H335: May cause respiratory irritation. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P280: Wear protective gloves/eye protection/face protection. P284: Wear respiratory protection. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. P362 + P364: Take off contaminated clothing and wash before reuse.
	DNA PBB (DNA Paraffin[b] Binding Buffer) (P/N: 05517621001) Tris-HCl buffer 49.6% Guanidine hydrochloride 0.05% Urea 17.3% Triton X-100	8 x 10 mL	
	WB I (DNA Wash Buffer I) (P/N: 05517656001) Tris-HCl buffer 64% Guanidine hydrochloride	1 x 25 mL	
	WB II (DNA Wash Buffer II) (P/N: 05517664001) Tris-HCl buffer Sodium chloride	1 x 12.5 mL	
	DNA EB (DNA Elution Buffer) (P/N: 05517630001) Tris-HCl buffer 0.09% Sodium azide	1 x 6 mL	
	HPEA FT (High Pure Extension Assembly Unit) (P/N: 07323204102) Filter tubes with caps	5 x 5 pcs	
	CT (Collection Tubes) (P/N: 05880513001)	3 x 25 pcs	

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® EGFR Mutation Test v2 Kit 24 Tests (P/N: 07248563190)	**EGFR MMX-1** (EGFR Master Mix 1) (P/N: 06471366001) Tris buffer Potassium chloride Glycerol EDTA Tween 20 3.13% Dimethyl sulfoxide 0.09% Sodium azide < 0.10% dNTPs < 0.01% Z05-AS1 DNA polymerase (microbial) <0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) < 0.01% Aptamer < 0.01% Upstream and downstream EGFR primers < 0.01% Fluorescent labeled EGFR probes	2 x 0.48 mL	N/A
	EGFR MMX-2 (EGFR Master Mix 2) (P/N: 06471382001) Tris buffer Potassium chloride Glycerol EDTA Tween 20 3.13% Dimethyl sulfoxide 0.09% Sodium azide < 0.10% dNTPs < 0.01% Z05-AS1 DNA polymerase (microbial) < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) < 0.01% Aptamer < 0.01% Upstream and downstream EGFR primers < 0.01% Fluorescent labeled EGFR probes	2 x 0.48 mL	N/A
	EGFR MMX-3 v2 (EGFR Master Mix 3) (P/N: 07248601001) Tris buffer Potassium chloride Glycerol EDTA Tween 20 3.13% Dimethyl sulfoxide 0.09% Sodium azide < 0.10% dNTPs < 0.01% Z05-AS1 DNA polymerase (microbial) < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial) < 0.01% Aptamer < 0.01% Upstream and downstream EGFR primers < 0.01% Fluorescent labeled EGFR probes	2 x 0.48 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® EGFR Mutation Test v2 Kit 24 Tests (P/N: 07248563190)	**MGAC** (Magnesium acetate) (P/N: 05854326001) Magnesium acetate 0.09% Sodium azide	6 x 0.2 mL	N/A
	EGFR MC (EGFR Mutant Control) (P/N: 06471455001) Tris buffer EDTA Poly-rA RNA (synthetic) 0.05% Sodium azide < 0.1% Plasmid DNA containing EGFR exon 18, 19, 20 and 21 sequences (microbial) < 0.1% EGFR wild-type DNA (cell culture)	6 x 0.1 mL	N/A
	DNA SD (DNA Specimen Diluent) (P/N: 05854474001) Tris-HCl buffer 0.09% Sodium azide	2 x 3.5 mL	N/A

[a] Product safety labeling primarily follows EU GHS guidance.

[b] Paraffin Binding Buffer is used for plasma samples.

Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® cfDNA Sample Preparation Kit	15°C to 30°C	Once opened, stable up to 8 uses over 90 days or until the expiration date indicated, whichever comes first
cobas® EGFR Mutation Test v2*	2°C to 8°C	Once opened, stable for 4 uses over 90 days or until the expiration date indicated, whichever comes first

Note: With the exception of the **PK** reagent, do not freeze reagents.

***EGFR MMX-1, EGFR MMX-2, EGFR MMX-3 v2,** and working MMX (prepared by the addition of **MGAC** to **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2**) should be protected from prolonged exposure to light. Working MMX must be stored at 2ºC to 8ºC in the dark. The prepared samples and controls must be added within 1 hour of preparation of the working MMX. Amplification must be started within 1 hour from the time that the processed samples and controls are added to the working MMX.

Additional materials required

Materials	P/N
Absolute ethanol (200 proof, for Molecular Biology)	Sigma E7023 or Fisher Scientific BP2818-500 or equivalent
Isopropanol (ACS, > 99.5%)	Sigma 190764 or Fisher Scientific A451-1 or equivalent
Sterile, nuclease-free water (for Molecular Biology)	Any vendor
Bleach	Any vendor
70% Ethanol	Any vendor
Sterile disposable, serological 5- and 25-mL pipettes	Any vendor
cobas® 4800 System Microwell Plate (AD-plate) and sealing film	Roche 05232724001
cobas® 4800 System sealing film applicator (supplied with the installation of the **cobas**® 4800 System)	Roche 04900383001
Adjustable pipettors* (Capable of pipetting 5 – 1000 µL)	Any vendor
Aerosol barrier or positive displacement DNase-free pipette tips	Any vendor
Pipet-Aid™*	Drummond 4-000-100 or equivalent
Table top centrifuge* (capable of 4,000 x g while holding 50-mL conical tubes in a swing bucket rotor)	Eppendorf model 5810 or equivalent
Bench top microcentrifuge* (capable of 20,000 x g)	Eppendorf 5430 or 5430R or equivalent
15-mL Sterile conical plastic tubes	Any vendor
Locking-lid microcentrifuge tubes (1.5-mL RNase/DNase free/ PCR grade)	Any vendor
Conical and microcentrifuge tube racks	Any vendor
Vortex mixer*	Any vendor
Disposable powder-free gloves	Any vendor

* All equipment should be maintained according to manufacturer's instructions.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas z 480 analyzer
cobas® 4800 System Control Unit with System Software version 2.1 or higher
EGFR Plasma P2 Analysis Package Software version 1.0 or higher
Barcode Reader ext USB
Printer

For more information regarding the materials sold separately, contact your local Roche representative.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay.

- For in vitro diagnostic use only
- Safety Data Sheets (SDS) are available upon request from your local Roche office.
- This test is for use with plasma NSCLC samples. Samples should be handled as if infectious using good laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories[12] and in the CLSI Document M29-A4.[13]
- **DNA PBB** contains Triton X-100, an irritant to mucous membranes. Avoid contact with eyes, skin, and mucous membranes.
- The use of sterile disposable pipettes and DNase-free pipette tips is recommended.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling samples and kit reagents.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

Note: *Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.*

Contamination

- Gloves must be worn and must be changed between handling samples and **cobas**® EGFR Test reagents to prevent contamination. Avoid contaminating gloves when handling samples.
- Gloves must be changed frequently to reduce the potential for contamination.
- Gloves must be changed before leaving DNA Isolation areas or if contact with solutions or a sample is suspected.
- Avoid microbial and ribonuclease contamination of reagents.
- The amplification and detection work area should be thoroughly cleaned before working MMX preparation. Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas. For example, pipettors and supplies used for DNA Isolation must not be used to prepare reagents for Amplification and Detection.
- It is highly recommended that workflow in the laboratory proceed in a uni-directional manner, completing one activity before proceeding to the next activity. For example, DNA isolation should be completed before starting amplification and detection. DNA isolation should be performed in an area separate from amplification and detection. To avoid contamination of the working master mix with DNA samples, the amplification and detection work area should be thoroughly cleaned before working master mix preparation.

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents from different kits or lots.
- Do not use disposable items beyond their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **DNA EB**, **MGAC**, **EGFR MMX-1**, **EGFR MMX-2**, **EGFR MMX-3 v2**, **EGFR MC**, and **DNA SD** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Spillage and cleaning

- **DNA PBB** and **WB I** contain guanidine hydrochloride. If liquid containing this buffer is spilled, clean with suitable laboratory detergent and water. If a spill occurs with potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the appropriate **cobas**® 4800 System - System Manual to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas z** 480 analyzer. Clean the **cobas z** 480 analyzer according to procedures described in the appropriate **cobas**® 4800 System - System Manual.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas z** 480 analyzer, consult the **cobas z** 480 analyzer Instrument Manual.

Sample collection, transport, and storage

Note: *Handle all samples as if they are capable of transmitting infectious agents.*

Sample collection and handling

The **cobas**® cfDNA Sample Preparation Kit has been developed for use with K2 EDTA Plasma samples.

Plasma should be separated from blood within 4 hours of collection and stored as indicated below until tested.

Sample transport, storage, and stability

Plasma samples can be transported frozen. Transportation of plasma samples must comply with country, federal, state, and local regulations for the transport of etiologic agents.[14]

Plasma specimens are stable for either:

Plasma Sample Storage Temperature	≤ -70°C	2°C to 8°C
Storage Time	Up to 12 months	Up to 3 days

Processed sample storage and stability

Processed sample (extracted DNA) is stable for one of the following:

Extracted DNA Storage Temperature	-15°C to -25°C	2°C to 8°C	15°C to 30°C
Storage Time	Up to 2 freeze thaws over 60 days	Up to 21 days	Up to 7 days

Extracted DNA should be used within the recommended storage periods or before the expiration date of the **cobas**® cfDNA Sample Preparation Kit used to extract the DNA, whichever comes first.

Prior to using extracted, stored DNA stocks, pulse vortex and centrifuge the elution tube containing the stock.

Test procedure

Running the test

Figure 4 cobas® EGFR Test workflow with cobas® cfDNA Sample Preparation kit

1	Start the system
2	Perform instrument maintenance
3	Remove samples and reagents from storage
4	Prepare samples for binding to column
5	Perform DNA isolation
6	Elute DNA
7	Create work order and print plate layout
8	Prepare amplification reagents
9	Load microwell plate with amplification reagents
10	Load microwell plate with sample
11	Seal microwell plate
12	Load microwell plate on the **cobas z** 480 analyzer
13	Start the run
14	Review results
15	With LIS: send results to LIS
16	Unload analyzer

Instructions for use

Note: *Only K2 EDTA Plasma samples are to be used with the* ***cobas***® *EGFR Test.*

Note: *Refer to the* ***cobas*** *z 480 analyzer Instrument Manual for detailed operating instructions for the* ***cobas*** *z 480 analyzer.*

Run Size

A single run can include from 1 to 30 samples (plus controls) per 96-well Microwell plate. When running more than 24 samples, multiple **cobas**® EGFR Test kits will be required.

The **cobas**® EGFR Test kit contains sufficient reagents for 8 runs of 3 samples (plus controls) for a maximum of 24 samples per kit.

Reagent preparation and storage

Prepare working reagents as shown in the table below prior to using the kit for the first time. Use a 5-mL serological pipette to dispense the water. Use 25-mL serological pipettes to dispense the ethanol. If the Proteinase K has already been reconstituted and frozen, thaw a sufficient number of aliquots to process the number of samples to be run.

Reagents	Reconstitution / Preparation
Proteinase K **(PK)**	Reconstitute **PK** by adding 4.5 mL of sterile water to the vial using a sterile, disposable 5-mL serological pipette. Mix by inverting the vial 5 to 10 times. Aliquot 1.1 mL of reconstituted **PK** into 1.5-mL microcentrifuge tubes and store at -20°C for up to 90 days or until the expiration date, whichever comes first. If the **PK** has already been reconstituted and frozen, thaw sufficient number of aliquots to process the number of samples to be run (250 µL of reconstituted **PK** is required for each sample).
Wash Buffer I **(WB I)**	Prepare working **WB I** by adding 15 mL of absolute ethanol to the bottle of **WB I**. Mix by inverting the bottle 5 to 10 times. Note on the bottle that ethanol has been added and the date. Store working **WB I** at 15°C to 30°C for up to 90 days or until the expiration date, whichever comes first.
Wash Buffer II **(WB II)**	Prepare working **WB II** by adding 50 mL of absolute ethanol to the bottle of **WB II**. Mix by inverting the bottle 5 to 10 times. Note on the bottle that ethanol has been added and the date. Store working **WB II** at 15°C to 30°C for up to 90 days or until the expiration date, whichever comes first.

All solutions stored at 15°C - 30°C should be clear. If a precipitate is present in any reagent, warm the solution to 37°C until the precipitate dissolves. Do not use until all precipitate has been dissolved.

DNA isolation procedure

1. Label a 15-mL conical tube for each plasma sample and a negative control. Sterile water can serve as a negative control and can be processed the same way as samples.
2. Vortex plasma, then transfer 2 mL of each plasma sample or negative control (sterile water) to a separate 15-mL tube.

Note: *A minimum of 2 mL of plasma is required to process a sample with the* ***cobas***® *cfDNA Sample Preparation Kit.*

3. Add 250 µL **PK** to each tube.
4. Add 2 mL of **DNA PBB** to each tube.
5. Mix the sample tubes containing **DNA PBB/PK** by inverting 3 to 5 times.
6. Incubate each tube at room temperature (15°C to 30°C) for 30 minutes.

Note: *During the incubation, prepare the required number of* ***HPEA FT*** *by labeling each* ***HPEA FT*** *with proper identification on the cap of each* ***HPEA FT****.*

Note: *Each sample will need one* ***HPEA FT****, three collection tubes (****CT****) and two elution tubes (1.5-mL microcentrifuge tubes).*

Note: *During the incubation, label the required number of elution tubes (1.5-mL microcentrifuge tubes) with sample identification information.*

7. Add 500 µL isopropanol and mix lysate by inverting 3 to 5 times.
8. Transfer all of the lysate into the appropriately labeled **HPEA FT**.
9. Using table top centrifuge with a swing bucket rotor, centrifuge **HPEA FT** at 4,000 x g for 5 minutes.

10. After centrifugation, remove the **HPEA FT** from the 50-mL conical collection tube. Place the **HPEA FT** onto a **CT**. Remove the larger locking clip by twisting and pulling it away from the assembly.
11. Remove the smaller locking clip from underneath the filter tube (**FT**) cap by pushing it up so that the seal is broken on both sides of the cap and then pulling it away from the assembly.
12. Remove the **HPEA** from the **FT** by tilting the extender away from the cap side of the **FT.**
13. Discard the flow-through from the **HPEA FT** into chemical waste and properly dispose of the unit.
14. Label the filter cap appropriately.
15. Add 500 µL working **WB I** to each **FT**.

*Note: Preparation of working **WB I** is described in the table in the **Reagent preparation** section.*

16. Use benchtop microcentrifuge for the rest of the protocol.
17. Centrifuge **FT/CT** units at 8,000 x g for 1 minute.
18. Place each **FT** onto a new **CT**. Discard the flow-through in each **CT** into chemical waste and properly dispose of old **CT**.
19. Add 500 µL working **WB II** to each **FT**.

*Note: Preparation of working **WB II** is described in the table in the **Reagent preparation** section.*

20. Centrifuge **FT/CT** units at 8,000 x g for 1 minute.
21. Place each **FT** onto a new **CT**. Discard the flow-through from the old **CT** into chemical waste and properly dispose of the old **CT**.
22. Centrifuge **FT/CT** units at 16,000 x g – 20,000 x g for 1 minute to dry the filter membrane.
23. Place the **FT** onto an elution tube (1.5-mL RNase/DNase-free microcentrifuge tube) pre-labeled with sample identification information and put an orientation mark on each tube. Discard any flow-through in each **CT** into chemical waste and properly dispose of the old **CT**.
24. Add 100 µL **DNA EB** to the center of the **FT** membrane without touching the **FT** membrane.
25. Incubate **FT** with elution tube at room temperature (15°C to 30°C) for 5 minutes.
26. Place the tubes in the centrifuge with the orientation marks facing outward. Centrifuge **FT** with elution tube at 8,000 x g for 1 minute to collect eluate into the elution tube (pre-labeled 1.5-mL RNase/DNase-free microcentrifuge tube). The eluate is the DNA stock.
27. Discard the **FT**.
28. Slowly remove 80 µL of DNA stock, being careful not to disrupt the pellet (which may not be visible). Transfer removed DNA stock to a second elution tube (1.5-mL RNase/DNase-free microcentrifuge tube) pre-labeled with sample identification information. Close the caps on the elution tubes. DNA stock is ready for PCR tests. Store DNA stock according to instructions in ***Sample transport, storage, and stability*** section.

Note: *If the pellet is disrupted, return the DNA stock to the original elution tube, cap the tube, then pulse vortex the tube and, with the orientation mark facing outward, centrifuge the tube at 8,000 x g for 1 minute to collect eluate and repeat Step 28 to remove 80 µL of DNA stock.*

Amplification and detection

Note: *To avoid contamination of working MMX with DNA samples, amplification and detection should be performed in an area separated from DNA Isolation. The amplification and detection work area should be thoroughly cleaned before working MMX preparation. For proper cleaning, all surfaces including racks and pipettors should be thoroughly wiped with 0.5% sodium hypochlorite solution followed by wiping with a 70% ethanol solution. Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.*

Instrument set-up

Refer to the **cobas z** 480 analyzer Instrument Manual for detailed instruction for the **cobas z** 480 set up.

Test order set-up

For detailed instructions on the EGFR workflow steps, refer to the **cobas**® 4800 System **cobas z** 480 analyzer Instrument Manual and Software Operator's Manual for the **cobas**® EGFR Mutation Test v2.

Generate a plate map with the position of all the samples and controls in the run. The Mutant Control is loaded into positions A01 – A03 on the plate. The Negative Control is loaded into positions B01 – B03 on the plate. Diluted samples are then added in sets of 3 columns, starting from C01 – C03 through H10 – H12, as shown in Figure 5.

Figure 5 Plate layout for the cobas® EGFR Test

Row / Column	01	02	03	04	05	06	07	08	09	10	11	12
A	MC MMX 1	MC MMX 2	MC MMX 3 v2	S7 MMX 1	S7 MMX 2	S7 MMX 3 v2	S15 MMX 1	S15 MMX 2	S15 MMX 3 v2	S23 MMX 1	S23 MMX 2	S23 MMX 3 v2
B	NEG MMX 1	NEG MMX 2	NEG MMX 3 v2	S8 MMX 1	S8 MMX 2	S8 MMX 3 v2	S16 MMX 1	S16 MMX 2	S16 MMX 3 v2	S24 MMX 1	S24 MMX 2	S24 MMX 3 v2
C	S1 MMX 1	S1 MMX 2	S1 MMX 3 v2	S9 MMX 1	S9 MMX 2	S9 MMX 3 v2	S17 MMX 1	S17 MMX 2	S17 MMX 3 v2	S25 MMX 1	S25 MMX 2	S25 MMX 3 v2
D	S2 MMX 1	S2 MMX 2	S2 MMX 3 v2	S10 MMX 1	S10 MMX 2	S10 MMX 3 v2	S18 MMX 1	S18 MMX 2	S18 MMX 3 v2	S26 MMX 1	S26 MMX 2	S26 MMX 3 v2
E	S3 MMX 1	S3 MMX 2	S3 MMX 3 v2	S11 MMX 1	S11 MMX 2	S11 MMX 3 v2	S19 MMX 1	S19 MMX 2	S19 MMX 3 v2	S27 MMX 1	S27 MMX 2	S27 MMX 3 v2
F	S4 MMX 1	S4 MMX 2	S4 MMX 3 v2	S12 MMX 1	S12 MMX 2	S12 MMX 3 v2	S20 MMX 1	S20 MMX 2	S20 MMX 3 v2	S28 MMX 1	S28 MMX 2	S28 MMX 3 v2
G	S5 MMX 1	S5 MMX 2	S5 MMX 3 v2	S13 MMX 1	S13 MMX 2	S13 MMX 3 v2	S21 MMX 1	S21 MMX 2	S21 MMX 3 v2	S29 MMX 1	S29 MMX 2	S29 MMX 3 v2
H	S6 MMX 1	S6 MMX 2	S6 MMX 3 v2	S14 MMX 1	S14 MMX 2	S14 MMX 3 v2	S22 MMX 1	S22 MMX 2	S22 MMX 3 v2	S30 MMX 1	S30 MMX 2	S30 MMX 3 v2

Where: MC= Mutant Control, NEG = Negative Control S# = sample ID, and MMX # corresponds to Master Mix Reagent 1, 2, or 3 v2.

Note: *Any given sample must be spread across three consecutive columns in one row in order to generate a response.*

Note: *Working Master Mix 1 must be loaded into column 01, 04, 07, and 10 on the plate. Working Master Mix 2 must be loaded into column 02, 05, 08, and 11 on the plate. Working Master Mix 3 v2 must be loaded into column 03, 06, 09, and 12 on the plate.*

Note: *Up to 30 samples can be loaded onto a single plate. If more than one reagent kit is required to process all of the samples on the plate, then the kits must all be from the same lot.*

Reaction set-up

Preparation of working master mix (MMX-1, MMX-2 and MMX-3 v2)

*Note: **EGFR MMX-1, EGFR MMX-2, EGFR MMX-3 v2**, and working MMX are light-sensitive and must be protected from prolonged exposure to light.*

*Note: Due to the viscosity of the **EGFR MMX** reagents and working MMX, pipette slowly to ensure all mix is completely dispensed from the tip.*

*Note: The **EGFR MMX-1, EGFR MMX-2,** and **EGFR MMX-3 v2** may appear light blue/purplish. This does not affect the performance of the reagent.*

Prepare three bulk working MMX, one containing **EGFR MMX-1**, one containing **EGFR MMX-2**, and the other containing **EGFR MMX-3 v2** in separate 1.5 mL microcentrifuge tubes.

1. Calculate the volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2** required for each working MMX using the following formula:

   Volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2** required = (Number of Samples + 2 Controls +1) x 20 µL

2. Calculate the volume of **MGAC** required for each working MMX using the following formula:

   Volume of **MGAC** required = (Number of Samples + 2 Controls +1) x 5 µL

Use Table 20 to determine the volume of each reagent needed for the preparation of working MMX based on the number of samples included in the run.

Table 20 Volumes of reagents needed for working MMX-1, working MMX-2 and working MMX-3 v2

		# of Samples*									
		1	2	3	4	5	6	7	8	9	10
MMX	20 µL	80	100	120	140	160	180	200	220	240	260
MGAC	5 µL	20	25	30	35	40	45	50	55	60	65
Total Vol. for Each Working MMX (µL)		100	125	150	175	200	225	250	275	300	325

* Volumes for # of Samples is based on the sum of the # Samples + 2 Controls + 1

3. Remove the appropriate number of **EGFR MMX-1, EGFR MMX-2, EGFR MMX-3 v2,** and **MGAC** vials from 2°C to 8°C storage. Vortex each reagent for 5 seconds and collect liquid at the bottom of the tube before use. Label a sterile microcentrifuge tube for working MMX-1, working MMX-2, and working MMX-3 v2.
4. Add the calculated volume of **EGFR MMX-1** or **EGFR MMX-2** or **EGFR MMX-3 v2** to their respective working MMX tube.
5. Add the calculated volume of **MGAC** to the working MMX tubes.
6. Vortex the tubes for 3 to 5 seconds to ensure adequate mixing.

Note: Samples and controls should be added to the microwell plate (AD-plate) within 1 hour after the preparation of the working MMXs.

*Note: Use only **cobas**® 4800 System Microwell Plate (AD-plate) and Sealing film.*

Preparation of plate

Note: If using stored DNA stocks, follow the instructions in ***Sample transport, storage and stability*** *section.*

1. Pipette 25 µL of working MMX into each reaction well of the microwell plate (AD-plate) that is needed for the run. Do not allow the pipettor tip to touch the plate outside the well.
    - Add working MMX-1 (containing **EGFR MMX-1**) to the microwell plate (AD-plate) wells in columns 01, 04, 07, and 10, as needed.
    - Add working MMX-2 (containing **EGFR MMX-2**) to the microwell plate (AD-plate) wells in columns 02, 05, 08, and 11, as needed.
    - Add working MMX-3 v2 (containing **EGFR MMX-3 v2**) to the microwell plate (AD-plate) wells in columns 03, 06, 09, and 12, as needed.
2. Pipette 25 µL of **EGFR MC** into wells **A01, A02,** and **A03** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.
3. Using a new pipettor tip, pipette 25 µL of **NEG** into wells **B01, B02,** and **B03** of the microwell plate (AD-plate); mix well using pipette to aspirate and dispense within the well a minimum of two times.

Note: Each run must contain ***EGFR MC*** *in wells* ***A01, A02*** *and* ***A03****, and* ***NEG*** *in wells* ***B01, B02****, and* ***B03*** *or the run will be invalidated by the* ***cobas z*** *480 analyzer.*

Note: Change gloves as needed to protect against sample-to-sample contamination and external PCR reaction tube contamination.

4. Using new pipettor tips for each sample DNA, add 25 µL of the first sample DNA to wells C01, C02, and C03 of the microwell plate (AD-plate), using a new tip for the addition of the sample DNA to each well; mix each well using a pipette to aspirate and dispense within the well a minimum of two times. Repeat this procedure for the DNA from each sample and follow the template in Figure 5 until all samples' DNA are loaded onto the microwell plate (AD-plate). Ensure that all liquid is collected at the bottom of the wells.

Note: Prior to using stored DNA stocks, pulse vortex and centrifuge the elution tube containing the stock.

5. Cover the microwell plate (AD-plate) with sealing film (supplied with the plates). Use the sealing film applicator to seal the film firmly to the microwell plate (AD-plate).
6. Confirm that all liquid is collected at the bottom of each well before starting PCR.

Note: Amplification and detection should be started within 1 hour after the addition of the first sample DNA dilution to the working MMX.

Starting PCR

Refer to the **cobas**® EGFR Operator's Manual for detailed instructions on the EGFR workflow steps. When the "Select test" pop-up window appears, select "EGFR Plasma P2" and click the "OK" button.

Results

Interpretation of results

Note: All run and sample validation is performed by the ***cobas****® 4800 software.*

Note: A valid test run may include both valid and invalid sample results.

For a valid run, sample results are interpreted as shown in Table 21.

Table 21 Result interpretation for the cobas® EGFR Test

Test Result	Mutation Result	Interpretation
Mutation Detected	Ex19Del S768I L858R T790M L861Q G719X Ex20Ins (More than one mutation may be present)	Mutation detected in specified targeted EGFR region.
No Mutation Detected (NMD)*	N/A	Mutation not detected in targeted EGFR regions
Invalid	N/A	Sample result is invalid. Repeat the testing of samples with invalid results following the instructions outlined in the "**Retesting of samples with invalid results**" section below.
Failed	N/A	Failed run due to hardware or software failure. Contact your local Roche office for technical assistance

* A "No Mutation Detected" result does not preclude the presence of a mutation in the targeted EGFR regions because results depend on concentration of mutant sequences, adequate sample integrity, absence of inhibitors, and sufficient DNA to be detected.

Retesting of samples with invalid results

1. If the run is invalid, there will be insufficient volume of extracted DNA for each sample to repeat Amplification and Detection. Repeat the entire test procedure for all samples, starting with DNA isolation.
2. If the run is valid but the sample is invalid, there will be insufficient volume of extracted DNA for each sample to repeat Amplification and Detection. Repeat the entire test procedure for the invalid sample, starting with DNA isolation.

Quality control and validity of results

One set of **cobas**® EGFR Test Mutant Control (**EGFR MC**) (wells **A01, A02** and **A03**) and negative control (**NEG**) (wells **B01, B02** and **B03**) for working MMX-1, working MMX-2, and working MMX-3 v2 are included in each run of up to 30 samples. A run is valid if the **EGFR MC** and the **NEG** are valid. If an **EGFR MC** or **NEG** is invalid, the entire run is invalid and must be repeated.

Mutant control

The **EGFR MC** result must be 'Valid'. If the **EGFR MC** results are consistently invalid, contact your local Roche office for technical assistance.

Negative control

The **NEG** result must be 'Valid'. If the **NEG** results are consistently invalid, contact your local Roche office for technical assistance.

Procedural limitations

1. Test only the indicated specimen types. The **cobas**® EGFR Test has been validated for use with NSCLC K2 EDTA Plasma samples.
2. The **cobas**® EGFR Test performance was verified using the **cobas**® cfDNA Sample Preparation Kit (Roche P/N: 07247737190).
3. Detection of a mutation is dependent on the number of copies present in the sample and may be affected by sample integrity, amount of isolated DNA, and the presence of interfering substances.
4. Reliable results are dependent on adequate transport, storage and processing. Follow the procedures in these Instructions for Use and in the **cobas**® EGFR Operator's Manual.
5. Pipetting from the bottom of the elution tube may disrupt the pellet and adversely affect test results.
6. The addition of AmpErase enzyme into the **cobas**® EGFR Test Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in these Instructions for Use are necessary to avoid contamination of reagents.
7. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
8. Only the **cobas z** 480 analyzer has been validated for use with this product. No other thermal cycler with real-time optical detection can be used with this product.
9. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to another, users perform method correlation studies in their laboratory to qualify technology differences.
10. The presence of PCR inhibitors may cause false negative or invalid results.
11. Though rare, mutations within the genomic DNA regions of the EGFR gene covered by the primers or probes used the **cobas**® EGFR Test may result in failure to detect the presence of a mutation in exons 18, 19, 20, and 21 (results of "No Mutation Detected").
12. The **cobas**® EGFR Test shows cross-reactivity (results of "Mutation Detected") to the exon 19 L747S mutation, a rare acquired mutation that may confer resistance to TKI treatment.[15]
13. Samples tested at high concentrations (> 10^5 copies/mL) may generate false results.
14. The **cobas**® EGFR Test was verified for use with 25 µL of DNA stock per reaction well. DNA stock input volumes lower than 25 µL per reaction well are not recommended.
15. The procedure described above must be followed to detect ≥ 100 copies of mutant DNA per mL of K2 EDTA plasma for the EGFR mutations in Table 3.
16. Samples with results reported as "No Mutation Detected" may harbor EGFR mutations not detected by the assay.
17. Consideration should be made for a "No Mutation Detected" result in plasma to reflex to or be confirmed by tissue testing.

Non-clinical performance evaluation

Analytical performance

The following data is intended to demonstrate the analytical performance of the **cobas**® EGFR Test.

Analytical sensitivity – limit of blank

To assess performance of the **cobas**® EGFR Test in the absence of template and to ensure that a blank sample does not generate an analytical signal that might indicate a low concentration of mutation, samples of healthy-donor K2 EDTA plasma EGFR wild-type specimens were evaluated. Using the analysis prescribed in the CLSI EP17-A2 guideline[16], the Limit of Blank was determined to be zero for all mutations.

Limit of detection using cell line DNA

Sheared cell line DNAs containing each of the seven mutation classes detected by the test were added to healthy-donor K2 EDTA plasma that is wild-type for EGFR. Serial dilutions were prepared and 24 replicates of each panel member were tested, using each of three **cobas**® EGFR Test kit lots.

Limit of Detection was determined for each the seven mutation classes detected by the test as the lowest concentration of DNA that gave an EGFR "Mutation Detected" rate of at least 95% for the targeted mutation. The results are shown in Table 22.

Table 22 Limit of detection of cobas® EGFR Test with K2 EDTA Plasma

EGFR Exon	EGFR Mutation Group	EGFR Nucleic Acid Sequence	Limit of Detection (copies/mL)	COSMIC ID[10]
18	G719X	2156 G>C	100	6239
19	Exon 19 Deletion	2235_2249del15	75	6223
20	T790M	2369 C>T	100	6240
	S768I	2303 G>T	25	6241
	Exon 20 Insertion	2307_2308insGCCAGCGTG	25	12376
21	L858R	2573 T>G	100	6224
	L861Q	2582T>A	30	6213

*Cell line DNA, mechanically sheared to an average size of 220bp, had a WT DNA background of approximately 100,000 copies/mL.

This study demonstrates that the **cobas**® EGFR Test can detect mutations in EGFR exons 18, 19, 20, and 21 with ≤ 100 copies of mutant DNA per mL of plasma using the standard input of 25 μL of DNA stock per reaction well.

Cross reactivity to other Exon 18, 19, 20, and 21 mutations

AURA Extension and AURA2 clinical trial specimens

The **cobas**® EGFR Test gave "Mutation Detected" results for the following EGFR mutations observed in the AURA Extension and AURA2 clinical trial specimens (Table 23). The AURA Extension study was used to supplement the specimens in the AURA2 cohort and increase the likelihood of detecting rare mutations in plasma. Analytical performance of the **cobas**® EGFR Test in detecting these mutations has not been evaluated.

Table 23 Mutations observed in the AURA Extension and AURA2 Trial determined to cross-react with the cobas® EGFR Test

Exon	Mutation Sequence	AA Change	COSMIC ID[15]
19	2254_2277del24	S752_I759delSPKANKEI	6256
	2236_2256>ATC	E746_S752>I	133190
	2239_2247delTTAAGAGAA	L747_E749delLRE	6218
	2239_2256>CAG	L747_S752>Q	Not Found
	2239_2264>GCCAA	L747_A755>AN	85891
	2240_2264>CGAGAGA	L747_A755>SRD	Not Found

Specificity – microorganism

Specificity of the **cobas**® EGFR Test was evaluated by testing *Staphylococcus epidermidis* at 1 x 10^6 colony forming units, which was found not to cross react or interfere with the **cobas**® EGFR Test when added to healthy-donor K2 EDTA plasma samples containing wild-type and mutant EGFR sequences.

Interference

Triglycerides (37 mM. CLSI recommended high concentration[17]), 0.2 g/L of bilirubin (unconjugated or conjugated, CLSI recommended high concentration[17]), and hemoglobin (1.5 g/L) have been shown not to interfere with the **cobas**® EGFR Test when the potentially interfering substance was added to healthy-donor K2 EDTA plasma samples containing wild-type and mutant EGFR sequences. Hemoglobin at a concentration of 2.0. g/L in plasma has been shown to interfere with the **cobas**® EGFR Test. Albumin at a concentration of ≥ 60 g/L (60 g/L, CLSI recommended high concentration[17]) may interfere with the **cobas**® EGFR Test.

The study results demonstrate that EDTA, Neupogen, and TARCEVA® do not interfere with the performance of the **cobas**® EGFR Test when the potentially interfering substance was added to healthy-donor K2 EDTA plasma samples containing wild-type and mutant EGFR sequences.

Clinical performance evaluation

Clinical reproducibility

A study was performed to assess the reproducibility of the **cobas**® EGFR Test across 3 testing sites (2 external and 1 internal, 2 operators per site), 3 reagent lots, and 3 non-consecutive testing days, with an nine-member panel of contrived samples consisting of cell-line DNA diluted in NSCLC plasma. Mutations including one exon 18 G719X mutation, one exon 19 deletion mutation, two exon 20 T790M mutations, one exon 20 insertion mutation, one exon 21 L858R mutation, and one exon 21 L861Q mutation, were represented in four contrived samples as summarized in Table 24. Each contrived sample was prepared at two levels: approximately 100 copies/mL and 300 copies/mL. These contrived samples were built into eight separate panel members along with a wild type control to make the nine-member panel.

Table 24 Contrived sample mutation combinations

Cell-Line DNA Combination 1	Cell-Line DNA Combination 2	Cell-Line DNA Combination 3	Cell-Line DNA Combination 4
Exon 19 Del	L858R	S768I	L861Q
T790M	T790M	G719A	Exon20 ins

Overall 37 runs were performed with 36 valid runs and one invalid run. A total of 648 panels (or 1224 mutations) were tested, of which 646 panels (or 1220 mutations) had valid results. There were no results of "Mutation Detected" in 72 valid tests of the wild type panel member, producing 100% agreement. Agreements vary for the mutation members: eight achieved 100% agreement, five > 97%, and one mutation (G719X) demonstrated a lower agreement at approximately 90%. Results for the overall agreement by mutations are presented in Table 25 below. The coefficient of variation (CV) was ≤ 12.8% in all mutant panel members. For the internal and Mutant controls, the overall CV was ≤ 1.5%. The CV was ≤ 0.89% between lots and ≤ 1.47% within-lot.

Table 25 Overall estimates of agreement by mutation member in reproducibility study

Mutation Member	Number of Valid Tests	Agreement N	Agreement % (95% CI)[a]
Wild Type - NA	72	72	100 (95.0, 100.0)
Exon 18 G719A - 100 Copies/mL	72	65	90.3 (81.0, 96.0)[b]
Exon 19 Deletion (2235_2249del15) - 100 Copies/mL	72	72	100 (95.0, 100.0)
Exon 20 Insertion (2307_2308ins9) - 100 Copies/mL	72	72	100 (95.0, 100.0)
Exon 20 S768I - 100 Copies/mL	72	72	100 (95.0, 100.0)
Exon 20 T790M - 100 Copies/mL	143	139	97.2 (93.0, 99.2)
Exon 21 L858R - 100 Copies/mL	71	70	98.6 (92.4, 100.0)
Exon 21 L861Q - 100 Copies/mL	72	72	100 (95.0, 100.0)
Exon 18 G719A - 300 Copies/mL	71	70	98.6 (92.4, 100.0)
Exon 19 Deletion (2235_2249del15) - 300 Copies/mL	72	72	100 (95.0, 100.0)
Exon 20 Insertion (2307_2308ins9) - 300 Copies/mL	72	72	100 (95.0, 100.0)
Exon 20 S768I - 300 Copies/mL	71	71	100 (94.9, 100.0)
Exon 20 T790M - 300 Copies/mL	144	142	98.6 (95.1, 99.8)
Exon 21 L858R - 300 Copies/mL	72	71	98.6 (92.5, 100.0)
Exon 21 L861Q - 300 Copies/mL	72	72	100 (95.0, 100.0)

[a] 95% CI = 95% exact binomial confidence interval.

[b] Lower agreement for this sample was due primarily to multiple missed calls (n = 6/24 replicates combined) occurring primarily at one of three sites

Note: Results were in agreement when a Mutant Type panel member had a valid result of “Mutation Detected” or when Wild Type panel member had a valid result of “No Mutation Detected”.

Note: The samples used in this study consisted of cell line DNA mechanically sheared to an average size of 220bp and had a WT DNA background of approximately 12,000 copies/mL.

Limit of detection (LOD) using NSCLC plasma specimens

A study was performed to confirm the LOD with NSCLC plasma specimens for three exon 19 deletions, one L858R mutation, and one T790M mutation using the **cobas**® EGFR Test across three testing sites (two external and one internal, two operators per site), three reagent lots, and two non-consecutive testing days, with an 11-member panel of NSCLC plasma specimens (five mutations each with two levels: 1X LOD and 2X LOD; plus WT). Overall 12 runs were performed (two replicates per run), and all runs were valid. A total of 264 tests were performed with the 11 panel members, of which 262 (99.2%) tests were valid. “Mutation Detected” results were not observed in 23 valid tests of the wild type panel member, producing 100% agreement. The percentage agreement for Exon 20 T790M-1 X LOD is 95.6%; and 100% for all other mutant panel members. The agreement estimates by panel member are summarized in Table 26 below. The coefficient of variation (CV) was < 7.0% in all mutant panel members.

Table 26 Agreement estimates by panel member

Panel Member	Number of Valid Tests	Agreement N	Agreement % (95% CI)[a]
Wild Type - NA	23	23	100 (85.2, 100.0)
Exon 19 Deletion 1 - 1 X LOD	24	24	100 (85.8, 100.0)
Exon 19 Deletion 1 - 2 X LOD	24	24	100 (85.8, 100.0)
Exon 19 Deletion 2 - 1 X LOD	23	23	100 (85.2, 100.0)
Exon 19 Deletion 2 - 2 X LOD	24	24	100 (85.8, 100.0)
Exon 19 Deletion 3 - 1 X LOD	24	24	100 (85.8, 100.0)
Exon 19 Deletion 3 - 2 X LOD	24	24	100 (85.8, 100.0)
Exon 20 T790M - 1 X LOD	24	23	95.8 (78.9, 99.9)
Exon 20 T790M - 2 X LOD	24	24	100 (85.8, 100.0)
Exon 21 L858R - 1 X LOD	24	24	100 (85.8, 100.0)
Exon 21 L858R - 2 X LOD	24	24	100 (85.8, 100.0)

[a] 95% CI = 95% exact binomial confidence interval.

Note: Results are included as agreement when a valid test of Mutant Type panel member has a result of mutation detected or when a valid test of Wild Type panel member has a result of 'no mutation detected'.

Note: Clinical specimens used in this study had a WT DNA background of approximately 24,000 copies/mL

Correlation to reference method using Phase III plasma samples from the ASPIRATION cohort

The analytical accuracy of the **cobas**® EGFR Test in detecting exon 19 deletion and L858R mutations was assessed by comparing with a validated next generation sequencing (NGS) platform using plasma specimens from patients with advanced NSCLC from one or more of the following studies (ASPIRATION Cohort): Genentech clinical studies G027821 (MetMab) and G027761 (MetLung) along with Roche clinical study ML25637 (ASPIRATION).

One hundred and twenty-eight plasma samples with a volume of 2 mL and with valid paired results from both the **cobas**® EGFR Test in plasma and an NGS method using plasma samples were included in the agreement analysis for the EGFR exon 19 deletion or L858R mutations. A total of 32 samples had MD and 95 had NMD results by the NGS method. The PPA between the **cobas**® EGFR Test in plasma and NGS in plasma was 87.5% (95% CI: 71.9%, 95.0%); the NPA between the **cobas**® EGFR Test and NGS was 96.8% (95% CI: 91.1%, 98.9%), as presented in Table 27.

Table 27 Comparison of the cobas® EGFR Test in plasma with NGS for the detection of the EGFR exon 19 deletion or L858R mutations

Measure of Agreement	Percent Agreement (N)	95% CI
Positive Percent Agreement (PPA)	87.5% (28/32)	71.9%, 95.0%
Negative Percent Agreement (NPA)	96.8% (92/95)	91.1%, 98.9%

Correlation between plasma and tissue samples by the cobas® EGFR Test for the detection of exon 19 deletion and L858R mutations using Phase III samples from ENSURE

The ENSURE study (YO25121) was a multicenter, open label, randomized Phase III study to evaluate the efficacy and safety of erlotinib versus gemcitabine/cisplatin as the first-line treatment for stage IIIB/IV non-small cell lung cancer (NSCLC) patients with exon 19 deletion or L858R mutations in the tyrosine kinase domain of epidermal growth receptor (EGFR) in their tumors. A total of 647 patients were screened, 601 patients had valid tissue EGFR result for exon 19 deletion and L858R Mutation from **cobas**® EGFR Test, and 217 patients were randomized in the study.

Five hundred and seventeen patients (86.0%, 517/601) had matched plasma samples and 441 patients had a plasma sample volume≥2.0 mL, i.e. the sample volume for which the **cobas**® EGFR Test in plasma was validated.

The correlation of plasma and tissue samples by the **cobas**® EGFR Test for detection of the exon 19 deletion and L858R mutation was evaluated both separately and in aggregate. A total of 431 samples with paired valid results from both tissue and plasma samples by **cobas**® EGFR Test were included in the agreement analysis. The positive percent agreement (PPA) between plasma and tissue sample was 76.7% (95% CI: 70.5% to 81.9%), the negative percent agreement (NPA) was 98.2% (95% CI: 95.4% to 99.3%), for the detection of exon 19 deletion and L858R mutation in aggregate as presented in Table 28. The PPA, NPA and OPA for detection of exon 19 deletion and L858R mutation separately are also presented in Table 28.

Table 28 Agreement between plasma samples and tissue samples by cobas® EGFR Test in the detection of Exon 19 deletion and L858R mutation

Mutation	Measure of Agreement	Percent Agreement (N)	95% CI
Aggregate	Positive Percent Agreement (PPA)	76.7% (161/210)	70.5%, 81.9%
	Negative Percent Agreement (NPA)	98.2% (217/221)	95.4%, 99.3%
	Overall Percent Agreement (OPA)	87.7% (378/431)	84.2%, 90.5%
Exon 19 Deletion	Positive Percent Agreement (PPA)	80.8% (97/120)	72.9%, 86.9%
	Negative Percent Agreement (NPA)	98.7% (307/311)	96.7%, 99.5%
	Overall Percent Agreement (OPA)	93.7% (404/431)	91.0%, 95.7%
L858R	Positive Percent Agreement (PPA)	67.8% (61/90)	57.6%, 76.5%
	Negative Percent Agreement (NPA)	99.1% (338/341)	97.4%, 99.7%
	Overall Percent Agreement (OPA)	92.6% (399/431)	89.7%, 94.7%

Note: PPA and NPA calculated using tissue as the reference.

Positive predictive value (PPV) and negative predictive value (NPV) for detection of exon 19 deletion and L858R mutations in aggregate were also calculated using the bootstrap method based on the different population tissue prevalence (Table 29). As expected, the PPV increases and NPV decreases as the EGFR mutation prevalence increases. For a Caucasian patient population, which assumes 10-15% tissue EGFR mutation prevalence, the PPV ranges from 82.8% to 88.6% while NPV ranges from 96.0% to 97.4%. The PPV ranges from 94.8% to 97.8% while NPV ranges from 80.8% to 90.9% if based on the prevalence in an Asian population, assuming 30-50% tissue EGFR mutation prevalence.

Table 29 Estimated predictive values of the cobas® EGFR Test in tissue and cobas® EGFR Test in plasma (patients with plasma sample volumes ≥ 2.0 mL) based on differing tissue EGFR mutation prevalence

Assumed EGFR Prevalence Based on Tissue Samples	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
10%	82.8% (71.3%, 93.7%)	97.4% (96.2%, 98.7%)
15%	88.6% (79.7%, 96.9%)	96.0% (94.3%, 97.6%)
20%	91.6% (85.0%, 97.8%)	94.4% (92.3%, 96.3%)
30%	94.8% (90.0%, 98.6%)	90.9% (88.4%, 93.4%)
40%	96.8% (93.0%, 99.4%)	86.4% (83.3%, 89.4%)
50%	97.8% (95.0%, 100.0%)	80.8% (77.4%, 84.8%)

Note: The 95% CIs were calculated from the bootstrap method.

Note: The result of 79 samples with a volume < 2.0 mL were treated as invalid in this analysis.

Note: PPV and NPV calculated using plasma as the reference.

Correlation to reference method using Phase II samples from AURA2

The clinical performance of the **cobas**® EGFR Test was assessed by comparing it with a validated next generation sequencing (NGS) platform using plasma specimens from patients with advanced NSCLC who were screened in the Phase II AURA2 trial of TAGRISSO™.

Of the 383 eligible patients, 344 patients had a plasma specimen available and were tested by the **cobas**® EGFR Test, with 342 valid results and two invalid results. Of a total of 344 plasma specimen tested by the **cobas**® EGFR Test, 322 (93.6%) were also tested by an NGS method and 22 did not have enough plasma volume remaining to be tested by NGS.

The analytical accuracy of the **cobas**® EGFR Test compared with the reference method, NGS, for detection of the T790M mutation in plasma samples was evaluated. A total of 320 samples with valid paired results from both **cobas**® EGFR Test and NGS results were included in the agreement analysis. The positive percent agreement (PPA) between the **cobas**® EGFR Test and NGS was 91.5% (95% CI: 85.7% to 95.1%), the negative percent agreement (NPA) was 91.1% (95% CI: 86.0% to 94.4%), for the detection of the T790M mutation as presented in Table 30.

Table 30 Comparison of the cobas® EGFR Test in plasma with NGS for the detection of the EGFR T790M mutation

Measure of Agreement	Percent Agreement (N)	95% CI
Positive Percent Agreement (PPA)	91.5% (129/141)	85.7%, 95.1%
Negative Percent Agreement (NPA)	91.1% (163/179)	86.0%, 94.4%

In plasma samples from the AURA2 trial, the **cobas**® EGFR Test detected the following mutations in exon 18, 19, 20 and 21 of the EGFR gene listed in Table 31.

Table 31 Mutations detected by the cobas® EGFR Test in the AURA 2 cohort

Exon	Mutation Sequence	AA Change	COSMIC ID[15]
18	2156G>C	G719A	6239
	2155G>A	G719S	6252
	2155G>T	G719C	6253
19	2235_2249del15	E746_A750delELREA	6223
	2236_2250del15	E746_A750delELREA	6225
	2236_2256>ATC	E746_S752>I	133190
	2237_2251del15	E746_T751>A	12678
	2237_2255>T	E746_S752>V	12384
	2237_2258>TATC	E746_P753>VS	Not Found
	2238_2248>GC	L747_A750>P	12422
	2239_2247delTTAAGAGAA	L747_E749delLRE	6218
	2239_2248TTAAGAGAAG>C	L747_A750>P	12382
	2239_2251>C	L747_T751>P	12383
	2239_2256>CAG	L747_S752>Q	Not Found
	2239_2256del18	L747_S752delLREATS	6255
	2239_2258>CA	L747_P753>Q	12387
	2239_2264>GCCAA	L747_A755>AN	85891
	2240_2251del12	L747_T751>S	6210
	2240_2254del15	L747_T751delLREAT	12369
	2240_2257del18	L747_P753>S	12370
	2240_2264>CGAGAGA	L747_A755>SRD	Not Found
	2253_2276del24	S752_I759delSPKANKEI	13556
20	2369C>T	T790M	6240
	2303G>T	S768I	6241
21	2573T>G	L858R	6224
	2573_2574TG>GT	L858R	12429
	2582T>A	L861Q	6213

Correlation between plasma and tissue samples for the detection of T790M using Phase II samples from AURA2

The AURA2 trial was a Phase II, open-label, single-arm study, assessing the safety and efficacy of TAGRISSO™ as a second or ≤ third-line therapy in patients with advanced NSCLC, who had progressed following prior therapy with an approved EGFR TKI agent and were T790M positive as determined by the **cobas**® EGFR Test. A total of 472 patients were screened, 383 patients had a tissue sample tested and 371 patients had a valid tissue EGFR result for the T790M mutation from the **cobas**® EGFR Test, of which 233 patients were T790M positive and 210 patients were randomized in the study.

Of the 383 eligible patients, 344 patients had plasma samples. A total of 334 samples with paired valid results from both tissue and plasma samples by the **cobas**® EGFR Test were included in the analysis. The positive percent agreement (PPA) between plasma and tissue samples was 58.7% (95% CI: 52.2%, 65.0%) and the negative percent agreement (NPA) was

80.2% (95% CI: 71.8%, 86.5%) for the detection of the T790M mutation. The positive predictive value (PPV) was 85.6% (95% CI: 79.2%, 90.3%) and the negative predictive value (NPV) was 49.2% (95% CI: 42.0%, 56.4%) for the detection of the T790M mutation as presented in Table 32.

The PPV shown in Table 32 was impacted by the 22 samples which were T790M negative by the **cobas**® EGFR Test in tissue and T790M positive by the **cobas**® EGFR Test in plasma. Eighteen samples were confirmed as T790M positive by NGS in plasma and one sample did not have enough volume for NGS testing. Only three were determined to be T790M negative by NGS.

Table 32 Agreement between plasma samples and tissue samples by the cobas® EGFR Test in the detection of the T790M mutation

Mutation	Measure of Agreement	Percent Agreement (N)	95% CI
T790M	Positive Percent Agreement (PPA)	58.7% (131/223)	52.2%, 65.0%
	Negative Percent Agreement (NPA)	80.2% (89/111)	71.8%, 86.5%
	Overall Percent Agreement (OPA)	65.9% (220/334)	60.6%, 70.8%
	Positive Predictive Value (PPV)	85.6% (131/153)	79.2%, 90.3%
	Negative Predictive Value (NPV)	49.2% (89/181)	42.0%, 56.4%

Note: PPA and NPA calculated using tissue as the reference.

Note: PPV and NPV calculated using plasma as the reference.

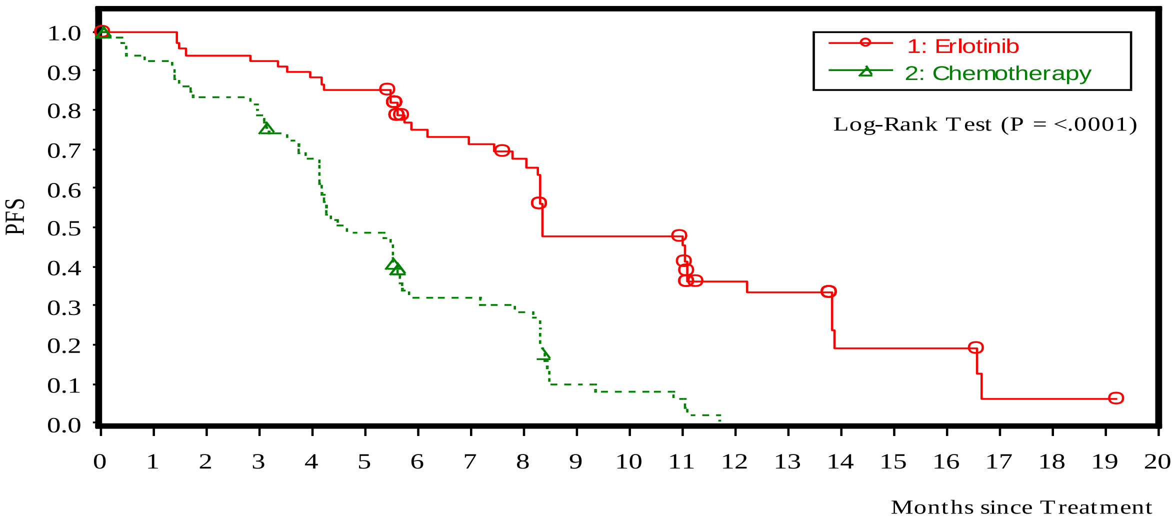
The agreement between plasma and tissue samples in the detection of the T790M resistance mutation is lower than for activating mutations. The PPA can be affected by tissue heterogeneity: unlike the activating mutations L858R and exon 19 deletions, T790M may be present in a small percentage of tumor cells as it is generally an acquired mutation; therefore, T790M cfDNA may only be present in very small amounts in plasma and below the level of detection. The NPA can also be affected by tumor heterogeneity: because the T790M mutation may not be present in all tumor cells, a tissue biopsy may be taken from a tumor in which the T790M mutation is not present while other tumor sites may be T790M-positive.[19]

Clinical outcome data

ENSURE

In the ENSURE trial, of the 217 patients enrolled (i.e., those with an exon 19 deletion or L858R mutation detected in a tissue sample by the **cobas**® EGFR Test v1), 214 (98.6%) had a plasma sample available and 180 patients had a plasma sample volume of2.0 mL. Of the 180 plasma samples with a volume of 2 mL tested by **cobas**® EGFR Test, 137 had a “Mutation Detected” result for an exon 19 deletion or an L858R mutation (68 patients in the erlotinib arm, 69 patients in the chemotherapy arm), 42 had a “No Mutation Detected” result (22 patients in the erlotinib arm, 20 patients in the chemotherapy arm), and one sample generated an invalid result. The Kaplan-Meier curves for the investigator assessed PFS are shown in Table 6. for patients with either an exon 19 deletion or L858R mutation in a plasma sample. The patients in the erlotinib arm had a longer PFS compared to patients in the chemotherapy arm and the two curves were well separated over the course of the observation period (p value < 0.001) showing substantial benefit to therapy with erlotinib in patients with a detectable EGFR activating mutation in plasma.

Figure 6 **Kaplan-Meier Plot of PFS by treatment for patients with mutation detected by the cobas® EGFR Test in both plasma and tissue (investigator assessment) (with 2 mL plasma samples)**

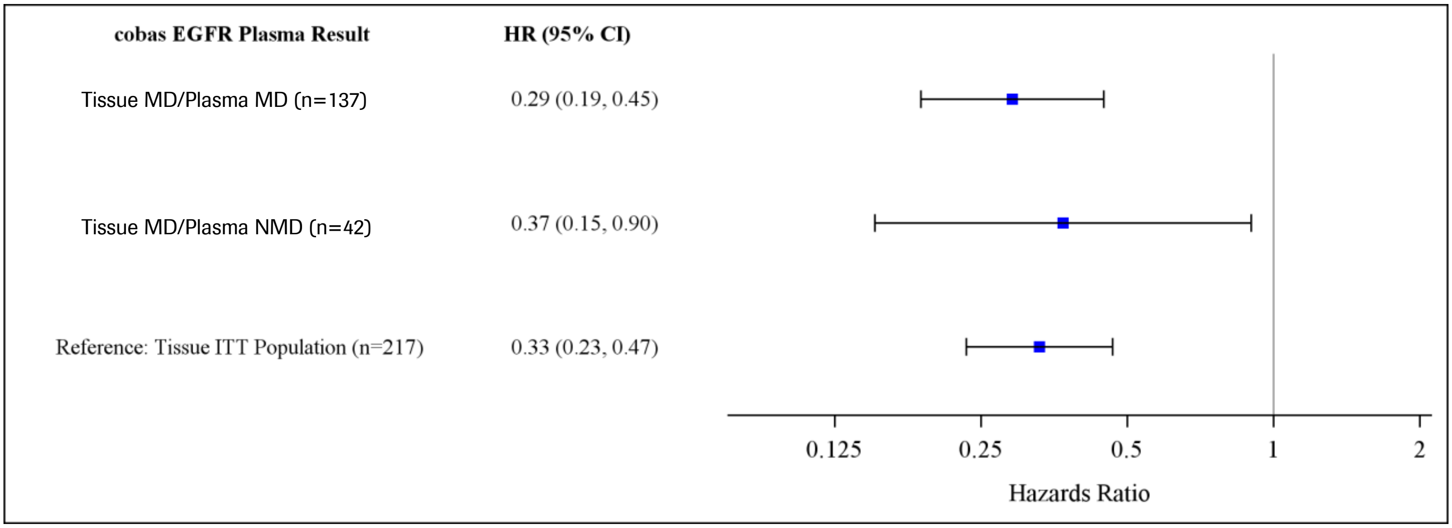


N at Risk

1	68	67	63	62	59	57	40	38	35	23	23	22	12	11	4	4	4	1	1	1	0
2	69	60	54	51	43	31	18	18	16	5	4	3	0								

A consistent PFS benefit was observed for all patients who were tissue EGFR mutation positive with plasma sample volumes of 2.0 mL whether they were plasma mutation positive or negative and this benefit was similar to the PFS benefit observed in the overall ENSURE ITT population (HR = 0.33; 95% CI: 0.23, 0.47) as shown in Figure 7 below.

Figure 7 **Forest plot for the HRs for PFS by investigator assessment (with 2 mL plasma samples)**



Note: MD = Mutation Detected (exon 19 deletion or L858R); NMD = No Mutation Detected (exon 19 deletion and L858R)

AURA2

The primary efficacy endpoint variable was the objective response rate (ORR) according to RECIST 1.1 by blinded independent central review (BICR) using the evaluable for response analysis set. The ORR was defined as the number (%) of patients with at least one visit and a result of complete response (CR) or partial response (PR) that was confirmed at least four weeks later (i.e., a best objective response [BOR] of CR or PR).

In the tissue Evaluable Response Analysis Set (ERAS) population (T790M+ patients by the **cobas**® EGFR Test in tissue who received at least one dose of TAGRISSO™ and had measurable disease at baseline according to BICR), 111 patients were plasma T790M+ by the **cobas**® EGFR Test (i.e. T790M+ by both the tissue and plasma samples). The ORR for this subset was 64.9% (72/111, 95% CI: 52.1%, 70.4%), which is very similar to the 64.1% observed ORR in the tissue ERAS population.

In the tissue Full Analysis Set (FAS) population (T790M+ patients by the **cobas**® EGFR Test in tissue who received at least one dose of TAGRISSO™), 117 patients were plasma T790M+ by the **cobas**® EGFR Test. The ORR for patients with a T790M+ result by both tissue and plasma samples was 61.5% (72/117, 95% CI: 55.2%, 73.7%), which is also very similar to the 61% observed ORR in the tissue FAS population. The results of these analyses are presented in Table 33. As enrollment in AURA2 was based on positive tissue test results, outcome data for (T790M plasma+, T790M tissue-) patients are not available from this trial.

Table 33: Objective response rate by plasma result among enrolled patients (T790M+ by tissue) from AURA2 study

Population (T790M+ by tissue sample)	Results of cobas® EGFR Mutation Test from Plasma Sample	N	Number of Patients with Response (ORR)[a] n(%)	ORR (95% CI)
Tissue Full Analysis Set (Tissue FAS)	T790M+(Plasma FAS)	117	72 (61.5%)	(55.2%, 73.7%)
	T790M-	89	53 (59.6%)	(51.3%, 73.0%)
	Overall (Tissue FAS)	210	128 (61.0%)	(57.0%, 70.8%)
Tissue Evaluable Response Analysis Set (Tissue ERAS)	T790M+(Plasma ERAS)	111	72 (64.9%)	(52.1%, 70.4%)
	T790M-	83	52 (62.7%)	(48.6%, 69.8%)
	Overall (Tissue ERAS)	198	127 (64.1%)	(54.0%, 67.6%)

[a] Responses include confirmed responses only.

Additional information

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 34 Symbols used in labeling for Roche PCR diagnostic products

SW	Ancillary Software	IVD	*In Vitro* Diagnostic Medical Device
EC REP	Authorized Representative in the European community	LLR	Lower Limit of Assigned Range
BARCODE	Barcode Data Sheet	🏭	Manufacturer
LOT	Batch code	🚫	Store in the dark
☣	Biological Risks	Σ	Contains Sufficient for <*n*> tests
REF	Catalogue number	🌡	Temperature Limit
ℹ	Consult instructions for use	TDF	Test Definition File
Cont.	Contents of kit	ULR	Upper Limit of Assigned Range
D	Distributed by	⌛	Use-by date
?	For IVD Performance Evaluation Only	GTIN	Global Trade Item Number

CE This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 35 Manufacturer and distributors

🏭	Manufactured in the United States Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany
D	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)

Trademarks and patents

See http://www.roche-diagnostics.us/patents

Copyright

References

1. Sharma SV, Bell DW, Settleman J, DA Haber. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007;7:169-181.
2. Pao W, J Chmielecki. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer. 2010;10:760-774.
3. Zhou C, Wu Y-L, G Chen. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011;12:735-742.
4. Paz-Ares L, Soulières D, I Melezínek. Clinical outcomes in non-small-cell lung cancer patients with EGFR mutations: pooled analysis. J Cell Mol Med. 2010;14:51-69.
5. Cheng L, Alexander RE, GT MacLennan. Molecular pathology of lung cancer: key to personalized medicine. Mod Path. 212; 25:347-369.
6. TARCEVA® (erlotinib) Package Insert.
7. Wu YL, Lee JS, S Thongprasert. Intercalated combination of chemotherapy and erlotinib for patients with advanced stage non-small-cell lung cancer (FASTACT-2): a randomized, double-blind trial. Lan Onc. 2013; 14:777-86.
8. TAGRISSO™ (osimertinib) Package Insert.
9. Janne PA, Yang JCH, DW Kim. AZD9291 in EGFR Inhibitor-Resistant Non-Small-Cell Lung Cancer. NEJM. 2015; 372:1689-99.
10. Catalogue of Somatic Mutations in Cancer (COSMIC), 2011, v.51. http://www.sanger.ac.uk/genetics/CGP/cosmic.
11. Longo MC, Berninger MS, JL Hartley. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene. 1990;93:125-128.
12. Chosewood LC, DE. Wilson. Biosafety and microbiological and biomedical laboratories-Fifth Edition. US Department of Health and Human Services Publication. (CDC). 2009;21-1112.
13. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4: Wayne, PA;CLSI, 2014
14. International Air Transport Association. Dangerous Goods Regulations, 57th Edition. 2016.
15. Costa DB, Nguyen KS, Cho BC, Sequist LV, Jackman DM, Riely GJ, Yeap BY, et al. Effects of erlotinib in EGFR mutated non-small cell lung cancers with resistance to gefitinib. Clin Cancer Res. 2008;14(21):7060-7067.
16. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - Second Edition. CLSI Document EP17-A2: Wayne, PA; CLSI, Jun 2012.
17. Clinical and Laboratory Standards Institute (CLSI). Interference testing in clinical chemistry; Approved Guideline–Second Edition. CLSI Document EP7-A2E Appendix D:Wayne, PA; CLSI, 2005.
18. Rosell R. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. The Lancet Oncology. 2012;13 v3:239-246.
19. TK Sundaresan. Detection of T790M, the Acquired Resistance EGFR Mutation, by Tumor Biopsy versus Noninvasive Blood-Based Analyses.

Document revision

Document Revision Information	
Doc Rev. 1.0 11/2015	First Publishing.
Doc Rev. 2.0 06/2016	Minor language clarifications for Section A: For Use with FFPET Samples Added Section B: For Use with Plasma Samples Please contact your local Roche Representative if you have any questions.
Doc Rev. 3.0 10/2016	Added AURA2 data to Section B: For Use with Plasma Samples Please contact your local Roche Representative if you have any questions.