Instructions For Use

LightMix® Digital EGFR G719X

TIB Cat.-No. 20-3009-32 Roche Cat.- No. 10 286 636 001

Kit with reagents for a maximum of 32 (HiSens plate) or 46 (Universal plate) digital PCR reactions

Detection Kit for EGFR G719X

1. Content, Storage and Expiry

Table 1: Kit components.

Vial	Cap Color	Description	Total Reactions	
1	**RED**	Parameter Specific Reagents (PSR) containing premixed and dried primers and probes	**32** HiSens plate	**46** Universal plate
Standards and Control DNA:				
1	**White/blue**	Positive Control (PC): 4-6 % Mutant (c.2156G>C, COSM6239) in wild type (WT) genomic DNA	**12**	

Storage:

Upon arrival, store kits at 4 °C to 25 °C. Dried kits are stable until lot-specific expiry date printed on the label.

Do not freeze dry reagents.
Store protected from light.

Open Vial Stability / On-board Stability:

Once dissolved, reagents can be stored at 4 °C to 8 °C for daily use for up to 60 days. Store protected from light. For long-term storage, keep reagents at -15 °C to -25 °C (until expiry date). Minimize repeat freeze-thaw cycles.

2. Recommended Additional Materials (Not Provided)

Reagents and consumables	**Cat. No., Manufacturer**
Digital LightCycler® 5x DNA Master	Roche Cat. No.: 09393544001, Roche Diagnostics
Water PCR grade	Roche Cat. No.: 03315932001, Roche Diagnostics
Recommended Restriction Enzyme (RE): MseI	Cat. No.: R0525S, New England Biolabs
Digital LightCycler® Universal Nanowell Plate	Roche Cat. No.: 09033696001, Roche Diagnostics
Digital LightCycler® High Sensitivity Nanowell Plate	Roche Cat. No.: 09033718001, Roche Diagnostics
Digital LightCycler® Partitioning Fluid	Roche Cat. No.: 08861536001, Roche Diagnostics
Digital LightCycler® System	
Digital LightCycler® Partitioning Engine	Roche Cat. No.: 09224777001, Roche Diagnostics
Digital LightCycler® Analyzer	Roche Cat. No.: 09274804001, Roche Diagnostics

3. Introduction

The LightMix® Digital EGFR G719X allows the detection of the G719A, G719C and G719S mutations in the EGFR gene (COSMIC IDs: COSM6253, COSM6252 and COSM6239) in genomic human DNA from a nucleic acid extract. The c.2155G>T, c.2155G>A and c.2156G>C mutations cause a glycine to cysteine, serine or alanine amino acid substitution, respectively.

4. Description

Using PCR methodology, an 81 bp fragment covering the EGFR G719X mutations is amplified with specific primers. The mutation site in the PCR fragment is analyzed using four labeled probes, three mutant and one wild-type, binding competitively. The mutant probes are FAM-labeled, and the wild type (WT) probe is HEX-labeled. The PC included in the kit contains 4-6 % synthetic mutant target (c.2156G>C) in wild-type genomic DNA and is equivalent to ~17 ng/µl when dissolved in 60 µl of PCR grade water. It is recommended to always include at least one PC and one No-Template Control (NTC; e.g. PCR grade water) in each run.

5. Assay Sensitivity

The assay has been experimentally verified using a spiked synthetic template into human genomic DNA background to a sensitivity of 0.05 % at 1 copies per partition (cpp). With non-synthetic samples the sensitivity may differ.

6. Sample Material and Extraction

A specific sample preparation was not tested during verification.

7. Assay Preparation and Protocol

7.1 Reagent Preparation

Preparation of Parameter-Specific Reagents (PSR)

1. Spin the premixed **PSR** tube at 10,000 RPM for 30 seconds.
2. Check that the pellet is located at the tube bottom.
3. To each **PSR** tube add **85 µl** of water, PCR grade.
4. Incubate for 20 seconds at room temperature.
5. Vortex for 10 seconds.
6. Spin the tubes to collect drops.

► The reconstituted **PSR** has a concentration of **20X**.

Preparation of Positive Control (PC)

1. Spin the premixed **PC** tube at 10,000 RPM for 30 seconds.
2. Check that the pellet is located at the tube bottom.
3. To each **PC** tube add **60 µl** of water, PCR grade.
4. Incubate for 20 seconds at room temperature.
5. Vortex for 10 seconds.
6. Spin the tubes to collect drops.

Please note: Opening the vials may cause contaminations of the work-space (aerosol).

► Use **5 µl** of **PC** per digital PCR reaction.

7.2 Sample Preparation

First prepare the samples and adjust them to the desired concentration before assembling the master mix reagent. Table 2 shows human gDNA input loading (ng to cpp).

Table *2*: Human gDNA input loading.

cpp	Universal Plate	High Sensitivity Plate
0.5	46 ng	33 ng
1	92 ng	66 ng
2	184 ng	132 ng

Please note: If running the assay at 0.5 cpp DNA input, it might be impossible to achieve 0.05 % analytical sensitivity due to the low number of mutant copies.

7.3 Preparation of the Master Mix Reagent

In the following protocol, the Digital LightCycler® 5x DNA Master and a restriction enzyme (MseI) are used in combination with the kit. Prepare the reaction mixture for each sample according to the table below (Table 3). Each run should include at least one **NTC** (e.g. PCR grade water) to demonstrate the absence of contamination and one **PC**. The preparation of the master mix reagent volume should be for n+1 samples, where n is the total number of samples, controls and NTC to be tested. This is to ensure adequate volume is available for input into each nanowell plate lane.

Table 3: Reaction mixture preparation for one sample

			Plate type	
			High Sensitivity *	**Universal**
Component	**Stock conc.**	**Final conc.**	**Volume per reaction µL**	**Volume per reaction µL**
Digital LightCycler® DNA Master	5X	1X	10.0	7.0
PSR	20X	1X	2.5	1.75
Restriction enzyme (MseI)	10 U/µl	5 U/rxn	0.5	0.5
Water (PCR-grade purity)	N/A	N/A	Variable**	Variable**
*Recommended plate type **Depending on sample volume Prepare master mix reagent without sample and dispense the corresponding volume to a tube/stripe				
Sample	N/A	N/A	Up to 37.0	Up to 25.75
Total Volume			50.0	35.0
Plate Input Volume			45.0	30.0

⚠ Mix thoroughly by vortexing for up to 5 seconds or by pipetting up and down 3 or more times

7.4 Preparation of the Reaction Mixtures

1. Mix the master mix reagent thoroughly, spin down and check for the absence of air bubbles in the reagent vial.
2. Dispense the corresponding volume of master mix reagent (depending on plate type and sample volume according to Table 3) for each sample to be analyzed into a suitable reaction tube or stripe.
3. **Mandatory:**
   - Add PCR grade water instead of sample to one tube/well, representing **NTC**
   - Add **5 µl** of **PC** (and water, if necessary, up to the required total reaction volume for the selected plate type) to one tube/well.
4. Add prepared **samples** to the remaining tubes/wells.
5. Close the tubes/stripes and mix thoroughly by vortexing for up to 5 seconds to ensure homogenous distribution of template in the reaction mixture. Or mix by pipetting up and down 3 or more times.
6. Centrifuge reaction mixtures before proceeding to plate loading.

7.5 Plate Loading and Partitioning

See manufacturer's instruction (Instructions for Use of the Digital LightCycler® 5x DNA Master and the Digital LightCycler® User Assistance.)

7.6 Instrument Programming

Program the Digital LightCycler® Analyzer Software according to the table below (Table 4). Select FAM and HEX as detection channels. Do not change the integration time (default: 1000 ms).

Table 4: PCR cycling program.

Step	Temperature °C	Time sec	Cycles
UNG activation	50	120	1
Denaturation	95	120	1
Amplification: *denaturation*	95	10	40
Amplification: *annealing/extension*	58	20	
Cooling	40	30	1

8. Result Analysis and Interpretation

In order to analyze data, clustering of the partitions needs to be performed. Refer to Clustering and Data Analysis Guide to choose the appropriate clustering parameters and analyze data.

9. Precautions and Warnings

- For research use only. Not for use in diagnostic procedures.
- The PSR reagent is light-sensitive and must be protected from prolonged exposure to light.
- Before using this product, read the operator/safety instructions in the instruments operator's manual.
- General precautions for the handling of samples and generic laboratory materials are required.
- Use personal protective equipment such as laboratory coats, gloves and eye protection when handling samples, consumables, and reagents.
- Use the instruction for use version valid for the kit in use (see kit label).
- All materials of human origin and related waste must be considered potentially infectious.
- The use of disposable filter tips is mandatory.
- Dispose of unused reagents and inactivate waste materials according to the current local guidelines.

10. References

Instrument manual: Digital LightCycler® User Assistance

11. Manufacturer and Contact Details

Report device observations, deviations and problems to your local Roche representative. Please report lot number(s) and a brief description.

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12. Version History

IFU ID	Change/Event	Date
v1.0	Initial Release	2024-07-03