cobas®

Rx Only

cobas® cfDNA Sample Preparation Kit

For in vitro diagnostic use

IVD

cobas® cfDNA Sample Preparation Kit 24 Tests M/N: 07247737190

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Intended use

The **cobas**® cfDNA Sample Preparation Kit is used for manual sample preparation to isolate circulating cell-free DNA (cfDNA) from plasma samples.

Principles of the procedure

Sample preparation

Plasma samples are processed and 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 a 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.

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 (M/N: 07247737190)	**PK** (Proteinase K) (M/N: 05860695102) Proteinase K, lyophilized[b]	2 x 100 mg	 **DANGER** H315: Causes skin irritation. H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. 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. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
cobas® cfDNA Sample Preparation Kit 24 Tests (M/N: 07247737190)	**DNA PBB** (DNA Paraffin[c] Binding Buffer) (M/N: 05517621001) Tris-HCl buffer 49.6% Guanidine hydrochloride[b] 0.05% Urea 20% Non-ionic detergent[b]	8 x 10 mL	 **DANGER** H302: Harmful if swallowed. H315: Causes skin irritation. H318: Causes serious eye damage. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P280: Wear protective gloves/ eye protection/ face protection. P301 + P312 + P330 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. Rinse mouth. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. 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/doctor. P332 + P313 If skin irritation occurs: Get medical advice/ attention. P501: Dispose of contents/ container to an approved waste disposal plant. 50-01-1 Guanidine, hydrochloride (1:1) 9002-92-0 Poly(oxy-1,2-ethanediyl), .alpha.-dodecyl-.omega.-hydroxy-

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® cfDNA Sample Preparation Kit 24 Tests (M/N: 07247737190)	**WB I** (DNA Wash Buffer I) (M/N: 05517656001) Tris-HCl buffer 64% Guanidine hydrochloride[b]	1 x 25 mL	 **WARNING** H302 + H332: Harmful if swallowed or if inhaled. H315: Causes skin irritation. H319: Causes serious eye irritation. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P264: Wash skin thoroughly after handling. P280: Wear protective gloves/ eye protection/ face protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P337 + P313: If eye irritation persists: Get medical advice/ attention. P501: Dispose of contents/ container to an approved waste disposal plant.
	WB II (DNA Wash Buffer II) (M/N: 05517664001) Tris-HCl buffer Sodium chloride	1 x 12.5 mL	N/A
	DNA EB (DNA Elution Buffer) (M/N: 05517630001) Tris-HCl buffer 0.09% Sodium azide	1 x 6 mL	N/A
	HPEA FT (High Pure Extension Assembly Unit) (M/N: 07323204102) Filter tubes with caps	5 x 5 pcs	N/A
	CT (Collection Tubes) (M/N: 05880513001)	3 x 25 pcs	N/A

[a] Product safety labeling primarily follows EU GHS guidance.
[b] Hazardous substance
[c] Paraffin Binding Buffer is used for plasma samples.

Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® cfDNA Sample Preparation Kit	15 – 30°C	Once opened and reconstituted, stable for 90 days or until the expiration date indicated, whichever comes first.

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

Note: *Before use, visually inspect each reagent to ensure that there are no signs of leakage. If there is any evidence of leaking, do not use that material for testing.*

Note: *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.*

Additional materials required

Materials	P/N
Absolute ethanol (200-proof for Molecular Biology)	Any vendor
Isopropanol (ACS, ≥ 99.5%)	Any vendor
Sterile, nuclease-free water (for Molecular Biology grade)	Any vendor
Bleach	Any vendor
70% Ethanol	Any vendor
Sterile disposable, serological 5- and 25-mL pipettes	Any vendor
Adjustable pipettors* (Capable of pipetting 5 – 1000 µL)	Any vendor
Aerosol barrier or positive displacement DNase-free pipette tips	Any vendor
Pipet-Aid™*	Any vendor
Table top centrifuge* (capable of 6,000 x g while holding 50-mL conical tubes in a swing-bucket rotor)	Any vendor
Benchtop microcentrifuge* (capable of 20,000 x g)	Any vendor
15-mL Sterile conical plastic tubes	Any vendor
Locking lid microcentrifuge tubes (1.5-mL sterile, RNase/DNase free/ PCR grade)	Any vendor
Vortex mixer*	Any vendor
Freezer capable of -25°C to -15°C storage	Any vendor
Conical and microcentrifuge tube racks	Any vendor
Disposable powder-free gloves	Any vendor

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

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 sample preparation kit.

- For in vitro diagnostic use only.
- Safety Data Sheets (SDS) are available upon request from your local Roche office.
- All samples should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological Laboratories[1] and in the CLSI Document M29-A4.[2]
- **DNA PBB** contains a non-ionic detergent which is an irritant to mucous membranes. Avoid contact with eyes, skin, and mucous membranes.

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.*

- The use of sterile disposable pipettes and DNase-free pipette tips is recommended.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- Inform your local competent authority about any serious incidents which may occur when using this assay.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling specimens 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**® cfDNA Sample Preparation Kit 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 specimen is suspected.
- Avoid microbial and ribonuclease contamination of reagents.
- 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.

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** contains 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.

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 EDTA anti-coagulated plasma samples.

Plasma should be separated from blood according to the **Sample collection, transport, and storage** section of the assay-specific Instructions for Use.

Sample transport, storage, and stability

Transportation of plasma samples must comply with country, federal, state, and local regulations for the transport of etiologic agents.[3]

Refer to the **Sample collection, transport, and storage** section of the assay specific Instructions for Use for storage recommendations.

Processed sample storage and stability

Refer to the **Sample collection, transport, and storage** section of the assay specific Instructions for Use for storage recommendations.

Extracted cfDNA 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.

Sample preparation procedure

Using the kit

Figure 1 **cobas**® cfDNA Sample Preparation Kit workflow

1	Remove samples and reagents from storage
2	Prepare sample for binding to column
3	Perform DNA isolation
4	Elute DNA

Instructions for use

Note: The cobas® cfDNA Sample Preparation Kit has been developed for use with EDTA anti-coagulated plasma samples.

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 specimens 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 specimens to be run (250 µL of reconstituted **PK** is required for each specimen).
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 - 30°C should be clear. If 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 specimen 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 specimen 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 specimen 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 specimen 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 (RT: 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 the **Sample transport, storage, and stability** section in the assay specific Instructions For Use.

Note: Pipetting from the bottom of the elution tube may disrupt the pellet and adversely affect test results.

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.

Additional information

Symbols

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

Table 1 Symbols used in labeling for Roche PCR diagnostic products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributor

Table 2 Manufacturer and distributor

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

Made in USA

Distributed by

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)[1]

[1] For USA only.

Trademarks and patents

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

Copyright

References

1. Chosewood LC, Wilson DE. Biosafety and microbiological and biomedical laboratories-Fifth Edition. US Department of Health and Human Services Publication. (CDC). 2009;21-1112.
2. 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.
3. International Air Transport Association. Dangerous Goods Regulations, 60th Edition. 2019.

Document revision

Document Revision Information	
Doc Rev 6.0 12/2021	Revised to comply with IVDR requirements. Updated the harmonized symbol page. Added **Technical support** section. Added Made in statement. Updated distributors addresses. Please contact your local Roche representative if you have any questions.

The summary of safety and performance report can be found using the following link:

https://ec.europa.eu/tools/eudamed