



cobas[®] cfDNA Sample Preparation Kit

For in vitro diagnostic use



cobas[®] cfDNA Sample Preparation Kit 24 Tests P/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 (P/N: 07247737190)	PK (Proteinase K) (P/N: 05860695102) Proteinase K, lyophilized ^b	2 x 100 mg	 <p>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.</p>

^aProduct safety labeling primarily follows EU GHS guidance.


^bHazardous substance

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning ^a
cobas® cfDNA Sample Preparation Kit 24 Tests (P/N: 07247737190)	DNA PBB (DNA Paraffin ^c Binding Buffer) (P/N: 05517621001) Tris-HCl buffer 49.6% Guanidine hydrochloride ^b 0.05% Urea 17.3% Triton X-100 ^b	8 x 10 mL	 <p>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. 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. P501: Dispose of contents/ container to an approved waste disposal plant.</p>

^aProduct safety labeling primarily follows EU GHS guidance.

^bHazardous substance

^cParaffin Binding Buffer is used for plasma samples.

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning ^a
cobas® cfDNA Sample Preparation Kit 24 Tests (P/N: 07247737190)	WB I (DNA Wash Buffer I) (P/N: 05517656001) Tris-HCl buffer 64% Guanidine hydrochloride ^b	1 x 25 mL	 <p>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.</p>
	WB II (DNA Wash Buffer II) (P/N: 05517664001) Tris-HCl buffer Sodium chloride	1 x 12.5 mL	N/A
	DNA EB (DNA Elution Buffer) (P/N: 05517630001) Tris-HCl buffer 0.09% Sodium azide	1 x 6 mL	N/A
	HPEA FT (High Pure Extension Assembly Unit) (P/N: 07323204102) Filter tubes with caps	5 x 5 pcs	N/A
	CT (Collection Tubes) (P/N: 05880513001)	3 x 25 pcs	N/A

^aProduct safety labeling primarily follows EU GHS guidance.

^bHazardous substance

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.

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 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™*	Drummond 4-000-100 or equivalent
Table top centrifuge* (capable of 6,000 x g while holding 50-mL conical tubes in a swing-bucket rotor)	Eppendorf model 5810 or equivalent
Benchtop microcentrifuge* (capable of 20,000 x g)	Eppendorf 5430 or 5430R or equivalent
15-mL Sterile conical plastic tubes	Any vendor
Microcentrifuge tubes (1.5-mL RNase/DNase free/ PCR grade)	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 assay.

- 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 good laboratory procedures such as those outlined in Biosafety in Microbiological Laboratories¹ and in the CLSI Document M29-A4.²
- **DNA PBB** contains Triton X-100, an irritant to mucous membranes. Avoid contact with eyes, skin, and mucous membranes.
- **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.

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.

- **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.
- 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 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.

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 K2-EDTA Plasma samples.

Plasma should be separated from blood within 8 hours of collection and stored as indicated below until tested.

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

Plasma specimens are stable for one of the following:

Plasma Sample Storage Temperature	≤ -70°C	-15°C to -25°C	2°C to 8°C	15°C to 30°C
Storage Time	Up to 12 months	Up to 12 months	Up to 3 days	Up to 1 day

Processed sample storage and stability

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

Extracted cfDNA 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 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.

Test procedure

Running the test

Figure 1 cobas[®] cfDNA sample preparation workflow

1	Obtain Plasma Sample
2	Prepare sample for binding to column
3	Perform DNA isolation
4	Elute DNA

Instructions for use

Note: Only K2 EDTA Plasma samples are to be used with the cobas[®] cfDNA Sample Preparation 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 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 instructions in the **Sample transport storage and stability** section.

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



Ancillary Software



In Vitro diagnostic medical device



Authorized representative
in the European community



Lower Limit of Assigned Range



Barcode Data Sheet



Manufacturer



Batch code



Store in the dark



Biological risks



Contains sufficient for $\langle n \rangle$ tests



Catalogue number



Temperature limit



Consult instructions for use



Test Definition File



Contents of kit



Upper Limit of Assigned Range



Distributed by



Use-by date



For IVD performance evaluation
only



Global Trade Item Number

Rx Only US Only: Federal law restricts this
device to sale by or on the order of a
physician.





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 2 Manufacturer and distributors

	<p>Manufactured in the United States</p> <p>Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany www.roche.com</p>	
	<p>Roche Diagnostics (Schweiz) AG Industriestrasse 7 6343 Rotkreuz, Switzerland</p> <p>Roche Diagnostics GmbH Sandhofer Strasse 116 68305 Mannheim, Germany</p> <p>Roche Diagnostics, SL Avda. Generalitat, 171-173 E-08174 Sant Cugat del Vallès Barcelona, Spain</p> <p>Roche Diagnostica Brasil Ltda. Av. Engenheiro Billings, 1729 Jaguaré, Building 10 05321-010 São Paulo, SP Brazil</p> <p>Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)</p>	<p>Roche Diagnostics 201, boulevard Armand-Frappier H7V 4A2 Laval, Québec, Canada Pour toute assistance technique, appeler le: 1-877-273-3433)</p> <p>Roche Diagnostics 2, Avenue du Vercors 38240 Meylan, France</p> <p>Distributore in Italia: Roche Diagnostics S.p.A. Viale G. B. Stucchi 110 20052 Monza, Milano, Italy</p> <p>Distribuidor em Portugal: Roche Sistemas de Diagnósticos Lda. Estrada Nacional, 249-1 2720-413 Amadora, Portugal</p>

Trademarks and patents

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

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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, 52nd Edition. 2011.

Document revision

Document Revision Information	
Doc. Rev. 4.0 09/2018	Updated specimen handling stability for use with plasma samples. Clarified note on handling DNA stock/eluate. Added Rx Only symbol and description to and updated descriptions of the harmonized symbol page. Please contact your local Roche Representative if you have any questions.