cobas® cfDNA Sample Preparation Kit

For in vitro diagnostic use

IVD

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

TABLE OF CONTENTS

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 DNA 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	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. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only outdoors or in a well-ventilated area. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/eye protection/face protection. P285: In case of inadequate ventilation wear respiratory protection. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P304 + P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER or doctor/physician. P330: Rinse mouth. P333 + P313: If skin irritation or rash occurs. Get medical advice/attention. P362: Take off contaminated clothing and wash before reuse. P403 + P233: Store in a well-ventilated place. Keep container tightly closed. P405: Store locked up.
	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	

[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 – 30°C	Once opened and reconstituted, the PK reagent is stable for up to 30 days or until the expiration date indicated, whichever comes first. Once opened and reconstituted, the remaining cfDNA Sample Preparation Kit reagents are stable for up 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)	Applied Biosystems (Ambion) AM9937 or GE Healthcare Hyclone™ SH3053801 or equivalent
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)	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)	Life Technologies AM12400 or Eppendorf 022364120 or equivalent
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 Laboratories[1] 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.
- Gloves must be changed frequently to reduce the potential for contamination.
- 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 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.

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 specimens.

Plasma should be isolated from blood within 4 hours of blood collection and stored at ≤ -70°C 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.[3]

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:

Extracted DNA Storage Temperature	-15°C to -25°C	2°C to 8°C	15°C to 30°C
Storage Time	Up to 1 freeze thaw over 60 days	Up to 7 days	Up to 1 day

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.

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 30 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 one elution tube (1.5-mL microcentrifuge tube).

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, 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. 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. 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. Vortex eluate prior to use.
27. Discard the FT. Close the caps on the elution tubes.
28. DNA stock is ready for PCR tests after vortexing. Store DNA stock according to instructions in the **Processed sample storage and stability** section.

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 <*n*> 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

 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

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

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

Roche Diagnostics
201, boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
Estrada Nacional, 249-1
2720-413 Amadora, Portugal

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

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