AmoyDx
艾德生物

CE IVD

AmoyDx® Circulating DNA Kit

(Spin Column)

Instructions for Use

REF 8.02.0004 24 tests/kit

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This importer information is only applicable for EU market

Version: V01

Intended Use

The AmoyDx® Circulating DNA Kit is specially designed for isolation and purification of DNA from peripheral blood (serum or plasma) and pleural effusion. The purified DNA is suitable for downstream applications such as real-time quantitative PCR (qPCR & ddPCR) and NGS.

Intended User

The AmoyDx® Circulating DNA Kit is intended to be used by laboratory professionals only.

Principle

The AmoyDx® Circulating DNA Kit provides silica-based membrane and special lysis buffer system for circulating DNA extraction effectively. Peripheral blood and pleural effusion samples are lysed with Buffer CDL and Digest Solution to release DNA. The lysate is mixed with isopropanol and DNA Tracer to collect DNA by centrifuging. Then the precipitates are dissolved in Buffer CDB, Buffer CDD and ethanol to provide appropriate binding conditions for DNA, then the mixture is applied to a Micro NA Spin Column, where the DNA binds to the membrane and impurities are removed with wash buffer. The DNA is eluted in Buffer CDE.

Kit Contents

This kit contains the following materials (Table 1):

Table 1 Kit Contents

Tube No.	Component	Symbol	Quantity
-	**Micro NA Spin Columns**	**Micro NA Spin Columns** **微量核酸吸附柱**	24 pcs ×1
-	**Collection Tubes (2 mL)**	**Collection Tubes (2 mL)** **2 mL 收集管**	48 pcs ×1
-	**Centrifugal Tubes (1.5 mL)**	**Centrifugal Tubes (1.5 mL)** **1.5 mL 离心管**	24 pcs ×1
-	**Centrifugal Tubes (10 mL)**	**Centrifugal Tubes (10 mL)** **圆底离心管**	24 pcs ×1
1	**Buffer CDL**	**Buffer CDL** **裂解液 CDL**	31 mL ×2
2	**Digest Solution**	**Digest Solution** **消化液 DPK**	6 mL ×1
3	**DNA Tracer**	**DNA Tracer** **DNA 助沉剂**	11 mL ×1
4	**Buffer CDB**	**Buffer CDB** **结合液 CDB**	12 mL ×1
5	**Buffer CDD**	**Buffer CDD** **助溶剂 CDD**	1.1 mL ×1
6	**Buffer CW1**	**Buffer CW1** **洗涤液 CW1**	13 mL ×1
7	**Buffer CW2**	**Buffer CW2** **洗涤液 CW2**	6.5 mL ×1
8	**Buffer CDE**	**Buffer CDE** **洗脱液 CDE**	1.8 mL ×3

Note:

1. ***Buffer CDL***, ***Buffer CDB*** *and* ***Buffer CW1*** *contain guanidine salt, not compatible with disinfectants containing bleach or acidic solutions.*
2. *For the first time use, add* ***19 mL*** *ethanol (96~100%) into* ***Buffer CW1*** *and mix thoroughly; add* ***19 mL*** *ethanol (96~100%) into* ***Buffer CW2*** *and mix thoroughly. Tick the check box on the bottle label.*
3. *If* ***DNA Tracer, Digest Solution, Buffer CDD*** *contains precipitates, dissolve them by mixing the solution upside down gently, avoid*

melting by heating.

4. *If other reagents contain precipitates, dissolve them by melting by heating.*

Storage and Stability

The shelf life of the kit is 12 months. The kit should be transported and stored dry at room temperature (10~30℃).

Additional Reagents and Equipment Not Supplied with Kit

1) Ethanol (96~100%).
2) Isopropanol (pre-cooled).
3) Water bath (60℃ adjustable).
4) Heating block (56℃ adjustable).
5) Microcentrifuge (1.5 mL rotor and 13,000×g adjustable).
6) Centrifuge (10 mL rotor and 10,000×g adjustable).
7) Vortexer (2,500 rpm adjustable)
8) Palm centrifuge.
9) Sterile, DNase-free pipet tips.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use. Strictly follow the instruction during operation.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- **Buffer CDL**, **Buffer CDB** and **Buffer CW1** contain guanidine salt, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions directly to the sample-preparation waste.** If the liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

Signal Word	**Warning**
Hazard Statements:	
H302+H332:	Harmful if swallowed or harmful if inhaled.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
Precautionary Statements	
P261:	Avoid breathing dust/fume/gas/mist/vapours/spray.
P264:	Wash skin thouroughly after handling.
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+P312:	IF INHALED: Remove victim to fresh air and Keep at rest in a position comfortable for breathing.
P305+P351+P388:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- If a spill contains potentially infectious reagents, clean the affected area with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
- DO NOT pipet by mouth.

Decontamination and Disposal

- Gloves should be worn and changed frequently when handling samples and reagents to prevent cross contamination.
- Use filtered pipette tips when handling samples and reagents to prevent contamination.
- All disposable materials are for one-time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Specimen Collection, Transport and Storage

Plasma (with anticoagulants such as citrate, or EDTA) or serum, pleural effusion stored at below -15℃ for no more than 2 years. Be sure to:

1) Peripheral blood and pleural effusion samples should be treated as infectious materials. Take caution in handling the samples.
2) Do not use heparin as anticoagulant, since heparin will inhibit PCR amplification and enzyme digestion.

Assay Procedure

Note:

- *For the first time use, please add **19 mL** ethanol (96~100%) into **Buffer CW1** and mix thoroughly; add **19 mL** ethanol (96~100%) into **Buffer CW2** and mix thoroughly. Mark them clearly.*
- *Before the DNA extraction, please check all the reagents without leakage and mix the reagents well.*
- *If DNA Tracer contains precipitate, dissolve it by mixing upside down gently; avoid melting by heating.*

1) Gently pipet 4 mL serum, plasma or pleural effusion to a clean 10 mL centrifugal tube.
2) Add 2.4 mL **Buffer CDL**, 210 µL **Digest Solution** into the tube, close the lid, and mix by vortexing more than 10 seconds.
3) Incubate at 60℃ for 15 min.
   ***Note:** we recommend incubation using water bath.*
4) Place the tube in ice for 5 min to cooling to room temperature, then briefly centrifuge for 5~10 seconds.
5) Add 400µL **DNA Tracer**, mix gently by pipetting up and down.
6) Add 3.3 mL pre-cooling isopropanol (under 4℃), close the lid, and mix by inverting the tube 20 times.
7) Centrifuge at 10,000×g for 5 min.
8) Remove the supernatant and residual solution by pipetting.
   ***Note:** Make sure the residual solution is removed thoroughly to prevent PCR inhibitors left.*
9) Add 470 µL **Buffer CDB** to the remaining precipitate, and then add 40 µL **Buffer CDD**, close the lid.
   ***Note:** discharge Buffer CDD under the liquid surface.*
10) Precipitate Dissolution: Place the tube on the vortexer, and vortex at 2,500 rpm for 15 min.
    Note:
    - *Extend the vortexing time if necessary to ensure the precipitate is completely dissolved.*
    - *If there is no available vortexer, please intermittently vortex the tube by using common vortexer to dissolve the precipitate.*
11) Add 250 µL ethanol (96~100%) into the tube and mix well by pipetting up and down. Close the lid and centrifuge at 500×g for 1 min.
12) Transfer the entire lysate to the Micro NA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 10,000×g for 30 seconds.
13) Discard the flow-through in collection tube.
14) Add 700 µL **Buffer CW1** to Micro NA Spin Column, centrifuge at 10,000×g for 30 seconds.
15) Discard the flow-through in collection tube.
16) Add 700 µL **Buffer CW2** to Micro NA Spin Column, centrifuge at 10,000×g for 30 seconds.
17) Discard the collection tube with flow-through.
18) Place the Micro NA Spin Column in a clean 2.0 mL collection tube. Centrifuge at 13,000×g for 1 min.
19) Discard the collection tube with flow-through.

20) Place the Micro NA Spin Column in a clean 1.5 mL centrifugal tube, incubate at 56℃ for 2 min using heating block.

Note: *keep the Micro NA Spin Column uncapped.*

21) DNA Elution: Apply 30~100 µL **Buffer CDE** to the center of the membrane. Close the lid and incubate at 56℃ for 2 min, and centrifuge at 13,000×g for 1 min.

Note:

- *Do not touch the membrane.*
- *If the eluent is more than 50 µL, please apply two times elution to get higher yield.*
  (Elute the DNA with equal volume of eluent for two times: apply 50 µL **Buffer CDE** to the center of the membrane first, incubate at 56℃ for 2 min, and centrifuge at 13,000×g for 1 min. Then apply 50 µL **Buffer CDE** to the center of the membrane again, incubate at 56℃ for 2 min, and centrifuge at 13,000×g for 1 min. The resulting eluted DNA is 100 µL.)

22) The eluted DNA is immediately ready for use, or stored at below -15℃.

Note: *Buffer CDE is only for elution and storage of DNA, NOT for other use.*

Performance Characteristics

The extraction efficacy of the kit was established by testing of six clinical serum, plasma, or pleural effusion samples.

- Extracted DNA: Mean A260 ≥ 0.1, and Mean A260/A280 ratio ≥ 1.6.

Limitations

1) The quality of extracted DNA is subject to the influence of such factors as sample source, sampling process, collection site, storage conditions.
2) Sample quality has a high impact on quality and amount of the purified DNA.

General Notes

If any serious incident has occurred during the use of this device or as a result of its use, please report it to the manufacturer and to your national authority.

References

1) Chevillard S. A method for sequential extraction of RNA and DNA from the same sample, specially designed for a limited supply of biological material. *Biotechniques.* 1993 Jul;15(1):22-4.

Symbols

EC REP	Authorized representative in the European Community/European Union	IVD	In Vitro Diagnostic Medical Device
	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use-by Date
Σ	Contains Sufficient for <n> Tests		Temperature Limit
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
COMP	Kit Components	Done?□	Tick the box after adding ethanol to the vial
ADD	Adding	EtOH	Ethanol
	Importer		

Revision History

Revision	Effective Date	Revision History
B1.0	2022-05-26	First edition
V01	2022-11-04	1. Add the symbol and information of importer; 2. Add revision history; 3. Move “effective date” from first page to last page; 4. Implementation of new coding rules.