



AmoyDx[®] Circulating DNA Kit (Spin Column)

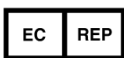
For purification of DNA from pleural effusion, serum or plasma

Instruction for Use

REF 8.02.26201X024G 24 tests



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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.

Principles of the Procedure

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 sufficient reagents to perform 24 tests (Table 1).

Table 1 Kit Contents

Component	Quantity	Tube No.
Micro NA Spin Columns	24 tubes	-
Collection Tubes (2 mL)	48 tubes	-
Centrifugal Tubes (1.5 mL)	24 tubes	-
Centrifugal Tubes (10 mL)	24 tubes	-
Buffer CDL	31 mL/vial ×2	1
Digest Solution	6 mL/vial ×1	2
DNA Tracer	11 mL/vial ×1	3
Buffer CDB	12 mL/vial ×1	4
Buffer CDD	1.1 mL/tube ×1	5
Buffer CW1 (concentrate)	13 mL/vial ×1	6
Buffer CW2 (concentrate)	6.5 mL/vial ×1	7
Buffer CDE	1.8 mL/vial ×3	8

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 (concentrate)** and mix thoroughly; add **19 mL** ethanol (96~100%) into **Buffer CW2 (concentrate)** and mix thoroughly. Tick the check box on the bottle label.
3. If **DNA Trace, 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. All components of the kit should be stored dry at room temperature (10~30°C).

Additional Reagents and Equipment Not Supplied With Kit

- 1) Ethanol (96~100%).
- 2) Isopropanol (pre-cooled).
- 3) Water bath (60°C adjustable).
- 4) Heating block (56°C 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, and 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.
- 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.
- Using 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.

Starting Material

Plasma (with anticoagulants such as citrate, or EDTA) or serum, pleural effusion stored at below -15°C 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 (concentrate)** and mix thoroughly; add **19 mL** ethanol (96~100%) into **Buffer CW2 (concentrate)** 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 μ L **Digest Solution** into the tube, close the lid, and mix by vortexing more than 10 seconds.
- 3) Incubate at 60°C 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 400 μ L **DNA Tracer**, mix gently by pipetting up and down.
- 6) Add 3.3 mL pre-cooling isopropanol (under 4°C), close the lid, and mix by inverting the tube 20 times.
- 7) Centrifuge at 10,000 \times 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 μ L **Buffer CDB** to the remaining precipitate, and then add 40 μ L **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 μ L ethanol (96~100%) into the tube and mix well by pipetting up and down. Close the lid and centrifuge at 500 \times 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 \times g for 30 seconds.
- 13) Discard the flow-through in collection tube.
- 14) Add 700 μ L **Buffer CW1** to Micro NA Spin Column, centrifuge at 10,000 \times g for 30 seconds.
- 15) Discard the flow-through in collection tube.
- 16) Add 700 μ L **Buffer CW2** to Micro NA Spin Column, centrifuge at 10,000 \times 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 \times 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°C for 2 min using heating block.
Note: keep the Micro NA Spin Column uncapped.
- 21) DNA Elution: Apply 30~100 μ L **Buffer CDE** to the center of the membrane. Close the lid and incubate at 56°C for 2 min, and centrifuge at 13,000 \times g for 1 min.
Note:
 - Do not touch the membrane.
 - If the eluent is more than 50 μ L, please apply two times elution to get higher yield.
(Elute the DNA with equal volume of eluent for two times: apply 50 μ L **Buffer CDE** to the center of the membrane first, incubate at 56°C for 2 min, and centrifuge at 13,000 \times g for 1 min. Then apply 50 μ L **Buffer CDE** to the center of the membrane again, incubate at 56°C for 2 min, and centrifuge at 13,000 \times g for 1 min. The resulting eluted DNA is 100 μ L.)
- 22) The eluted DNA is immediately ready for use, or stored at below -15°C.
Note: Buffer CDE is only for elution and storage of DNA, NOT for other use.

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.

Symbols



Authorized Representative in the European Community



Manufacturer



Batch Code



Contains Sufficient for <n> Tests



Consult Instructions For Use



This Way Up



In Vitro Diagnostic Medical Device



Catalogue Number



Use By



Temperature Limitation



Keep Dry



Fragile, Handle With Care