AmoyDx® Tissue DNA Kit

(Spin Column)

Instructions for Use

REF 8.02.0078 36 tests/kit

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This importer information is only applicable for EU market

Version: V01

Intended Use

The AmoyDx® Tissue DNA Kit is specially designed for isolation and purification of DNA from human tissue or pleural effusion precipitation. The purified DNA is suitable for the downstream PCR amplification, genotype analysis, restriction enzyme digestion and other experiments.

Intended User

The AmoyDx® Tissue RNA Kit is intended to be used by laboratory professionals only.

Principles of the Procedure

This kit uses efficient tissue lysis buffer system, combined with silicone membrane adsorption column technology, isolate and purify the genomic DNA from human tissue or pleural effusion precipitation.

Kit Contents

This kit contains sufficient reagents to perform 36 tests (Table 1).

Table 1 Kit Contents

Tube No.	Component	Symbol	Quantity
—	**DNA Spin Columns**	DNA Spin Columns DNA 吸附柱	36 pcs ×1
—	**Collection Tubes (2 mL)**	Collection Tubes (2 mL) 2 mL 收集管	72 pcs ×1
—	**Centrifugal Tubes (1.5 mL)**	Centrifugal Tubes (1.5 mL) 1.5 mL 离心管	72 pcs ×1
1	**Buffer DTL**	Buffer DTL 裂解液 DTL	10 mL ×1
2	**Proteinase K Solution**	Proteinase K Solution 蛋白酶 K 溶液	900 µL ×1
3	**Buffer DTB**	Buffer DTB 结合液 DTB	10 mL ×1
4	**Buffer DW1**	Buffer DW1 洗涤液 DW1	13 mL ×1
5	**Buffer DW2**	Buffer DW2 洗涤液 DW2	6 mL ×1
6	**Buffer DTE**	Buffer DTE 洗脱液 DTE	8 mL ×1

Note:

1) ***Buffer DTB*** *and* ***Buffer DW1*** *contain guanidine salt, not compatible with disinfectants containing bleach or acidic solutions.*
2) *For the first time use, add 17 mL ethanol (96~100%) into* ***Buffer DW1*** *and mix thoroughly; add 24 mL ethanol (96~100%) into* ***Buffer DW2*** *and mix thoroughly. Tick the check box on the bottle label.*

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 Required but Not Supplied

1) Ethanol (96~100%).
2) RNase A (optional).
3) Tissue preservation solution.
4) Water bath or heated orbital incubator (90℃ adjustable).
5) Microcentrifuge (10000~12000 ×g adjustable).

6) Vortexer.
7) Palm centrifuge.
8) 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 DTB*** and **Buffer DW1** 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.
- If a spill contains potentially infectious reagents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent cross-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

Rich DNA enzymes present in tissue. In the process of grinding and nucleic acid separation, DNA is readily biodegradable. Before using this kit to do DNA separation, be sure to:

1) Removed fresh tissue sample should be immediately stored in 4 to 8-fold volume of tissue preservation solution. Store and use the sample according to requirements and procedure.
2) Tissue sample should be stored at -70℃ for no more than 3 years.
3) If use other tissue preservation solution, wash the tissue sample with normal saline solution before the operation of DNA isolation.

Assay Procedure

1. Sample pretreatment

1.1 For tissue sample: transfer 20~80 mg of ground tissues into a clean 1.5 mL centrifuge tube, add 230 µL **Buffer DTL**.

Note:

- *Take proper amount of tissue sample like a grain of rice. The deficiency or excess of tissue amount may affect the kit performance.*
- *Tissues can be treated by liquid nitrogen grinding or shearing. Grinding thoroughly makes tissue lysis easier.*

1.2 For pleural fluid or cytology sample: take 10~50 mL of pleural fluid sample or appropriate cell suspension, centrifuge at 3000 ×g for 10 min and remove the supernatant by pipetting. Transfer 20~80 mg precipitation into a clean 1.5 mL centrifuge tube, add 230 µL **Buffer DTL**.

2. DNA Extraction

Note:

- *For the first time use, please add 17 mL ethanol (96~100%) into **Buffer DW1**, add 24 mL ethanol (96~100%) into **Buffer DW2**, and mark it clearly.*
- *Before the DNA extraction, please check the reagents without leakage. Shake the reagents gently to mix the solutions. If the reagents contain precipitates, dissolved by heating at 50 ℃.*

2.1 Add 20 µL **Proteinase K Solution** into the above centrifuge tube containing sample, mix by vortexing for 30 seconds, and then re-suspend the tissue.

Note: *Completely disrupt the precipitate by pipetting up and down if necessary.*

2.2 Briefly centrifuge the centrifuge tube and incubate at corresponding condition according to the sample type:

A. For surgery tissue sample: incubate at 90℃ for 45 min or 56℃ overnight.

B. For biopsy tissue sample: incubate at 90℃ for 20 min.

C. For pleural fluid or cytology sample: incubate at 56℃ for 1 hour.

Note: *if the thermomixer is available, incubate at 500 rpm in the thermomixer.*

2.3 Take out the sample tube, briefly centrifuge for a few seconds.

Note:

- *With the high temperature, be cautious of tube cap bounce off.*
- *If RNA-free genomic DNA is required, allow the sample to cool to room temperature, add 2 µL RNase A (100 mg/mL), mix by vortexing for 5 seconds, briefly centrifuge and incubate for 5 min at room temperature.*

2.4 Add 250 µL ethanol (96~100%), and then add 250 µL **Buffer DTB**, mix by vortexing for 10 seconds and centrifuge at 12000 ×g for 1 min.

2.5 Transfer the entire lysate to the DNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 12000 ×g for 1 min. Discard the flow-through in collection tube.

Note: *Don't touch the membrane.*

2.6 Add 600 µL **Buffer DW1** to DNA Spin Column, centrifuge at 12000 ×g for 30 seconds. Discard the flow-through in collection tube.

2.7 Add 600 µL **Buffer DW2** to DNA Spin Column, centrifuge at 12000 ×g for 30 seconds. Discard the collection tube with flow-through.

2.8 Place the DNA Spin Column in a clean 2 mL collection tube, centrifuge at 12000 ×g for 3 min. Discard the collection tube with flow-through.

2.9 Place the DNA Spin Column in a clean 1.5 mL centrifugal tube. Open the tube and incubate at 56℃ for 3 min. Make sure all residual ethanol has evaporated before proceeding.

2.10 Apply 30~100 µL **Buffer DTE** to the center of the membrane. Do not touch the membrane. Open the tube and incubate at 56℃ for 2 min. Centrifuge at 12,000 ×g for 1 min.

Note: *Two times elution makes for higher DNA yield. (e.g. If the elution volume is 100 µL, firstly apply 50 µL Buffer DTE to the center of the membrane, incubate at 56 ℃ for 2 min and centrifuge at 12,000 ×g for 1 min. Then apply another 50 µL Buffer DTE to the center of the membrane, incubate at 56 ℃ for 2 min and centrifuge at 12,000 ×g for 1 min.)*

2.11 The eluted DNA is immediately ready for use. If the DNA is not used within 2 hours, it should be stored at -20℃..

Note: Buffer DTE 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 tissue, pleural fluid, or cytology samples.

- Extracted DNA: Mean A260 ≥ 0.2, 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.