AmoyDx
艾德生物

CE IVD

AmoyDx® Tissue RNA Kit

(Spin Column)

For purification of RNA from human tissue or pleural effusion precipitation

Instructions for Use

REF 8.02.0079 36 tests/kit

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This importer information is only applicable for EU market

Version: V02

Intended Use

The AmoyDx® Tissue RNA Kit is specially designed for isolation and purification of RNA from human tissue or pleural effusion. The purified RNA is suitable for the downstream PCR amplification, Northern marking hybridization and other experiments.

Intended User

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

Principle

Tissue sample are lysed with Buffer RLB and Proteinase K solution to release RNA. Then the lysate is mixed with ethanol to provide appropriate binding conditions for RNA, then the mixture is applied to a RNA spin column, where the RNA binds to the membrane and impurities are removed with wash buffer. The RNA is eluted in Buffer RTE.

Kit Contents

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

Table 1 Kit Contents

Tube No.	Component	Symbol	Quantity
—	**RNA Spin Columns**	RNA Spin Columns RNA 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	54 pcs ×2
1	**Buffer RLB**	Buffer RLB 裂解结合液	31 mL×1
2	**Proteinase K Solution**	Proteinase K Solution 蛋白酶 K 溶液	900 µL ×1
3	**DNase I Magic Buffer**	DNase I Magic Buffer DNase I 工作液	1.5 mL ×1
4	**DNase I (30 U/µL)**	DNase I DNA 消化酶	40 µL ×1
5	**RNase-free Water**	RNase-free Water 无核酸酶水	1.5 mL ×1
6	**Wash Buffer A**	Wash Buffer A 洗涤液 A	13 mL ×2
7	**Wash Buffer B**	Wash Buffer B 洗涤液 B	6 mL ×2
8	**RNA Protection Buffer**	RNA Protection Buffer RNA 保护液	200 µL ×1
9	**Buffer RTE**	Buffer RTE 洗脱液	1.5 mL ×3
10	**Buffer TC**	Buffer TC 样本清洗液 TC	15 mL ×1

Note:

1) ***Buffer RLB*** *and* ***Wash Buffer A*** *contain guanidine salt, not compatible with disinfectants containing bleach or acidic solutions.*
2) *For the first time use, add 17 mL and 24 mL of absolute ethanol respectively into* ***Wash Buffer A*** *and* ***Wash Buffer B****, mix each of them thoroughly. Tick the check box on the bottle label.*
3) *For the first time use, please add 360 µL* ***RNase-free Water*** *into* ***DNase I (30 U/µL)*** *tube to obtain* ***DNase I (3 U/µL)*** *solution, and mix well by pipetting gently up and down. Store it at 4 ℃.*

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) Thermomixer with block for 1.5 mL tube (56℃ adjustable and 500 rpm adjustable).
3) Microcentrifuge (12,000 ×g adjustable).
4) Vortexer.
5) Palm centrifuge.
6) Sterile, RNase-free pipet tips.

Precautions and Handling Requirements

For *in vitro* diagnostic use.

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 RLB*** and **Wash Buffer A** 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.

Starting material

RNA in human tissue or pleural effusion precipitation is readily biodegradable in the process of grinding and nucleic acid separation. Before using this kit to do RNA separation, be sure to:

1) Removed fresh tissue sample should be immediately stored in 4~8 times volume of tissue preservation solution. Store and use the sample according to requirements and procedure.
2) Tissue sample's storage time should be less than three years at -70℃.
3) For the tissue samples which have been stored in liquid nitrogen or at -70℃, please add 10-fold volume of tissue protective solution before isolating, and thaw at 4℃.
4) If use other tissue protective solution, wash the tissue sample with Buffer TC before the operation of RNA isolation.

Additional Requirements for Handling of RNA

Observe the following guidelines to prevent RNase contamination and maximize the RNA yield:

1) Use disposable, sterile plastic ware.

2) Use sterile, new pipette tips and microcentrifuge tubes.

3) The glass or ceramic containers such as mortar, grinding rod which will be used during the operation should be soaked in 0.1% diethyl (DEPC) solution for 8 hours and baked at 180℃ for 4 hours for autoclaving.

4) Wear latex or nitrile gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin.

5) Use proper microbiological aseptic technique when working with RNA.

6) Use proper method to remove RNase contamination from surfaces.

Assay Procedure

1. Sample pretreatment

1.1 Pleural effusion samples:

1.1.1 Take 10~40 mL of pleural fluid samples, centrifuge at 3,000 ×g for 10 min and remove the supernatant by pipetting.

1.1.2 Place 20~80 mg precipitation into a clean 1.5 mL centrifugal tube, add 300 µL **Buffer TC** and mix by vortexing, then Centrifuge at 3,000 ×g for 10 min and remove the supernatant by pipetting.

1.2 Human tissue samples:

1.2.1 Transfer 20~60 mg of ground tissues into a clean 1.5 mL centrifuge tube.

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.*

2. RNA Extraction

Note:

- *For the first time use, add 17 mL and 24 mL of absolute ethanol respectively into **Wash Buffer A** and **Wash Buffer B**, mix each of them thoroughly, and mark it clearly.*
- *For the first time use, please add 360 µL **RNase-free Water** into **DNase I (30 U/µL)** tube to obtain **DNase I (3 U/µL)** solution, and mix well by pipetting gently up and down. Store it at 4℃.*
- *Before the RNA 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 800 µL **Buffer RLB** and 20 µL **Proteinase K Solution** into the above centrifuge tube containing sample, mix by vortexing for 15 seconds. Briefly centrifuge and incubate at 56℃ for 20 min at 500 rpm in the thermomixer.

2.2 Take out the centrifuge tube and cool to room temperature. Centrifuge at 12,000 ×g for 3 min. Transfer 800 µL of supernatant by pipetting slowly from top to down into a clean 1.5 mL centrifugal tube.

Note: *Do not touch the precipitate.*

2.3 Add 600 µL **ethanol** (96~100%) to the supernatant, close the lid and mix the solution by inverting the tube for 20 times.

2.4 Briefly centrifuge the tube and transfer 700 µL of lysate to the RNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 12,000 ×g for 30 seconds. Discard the flow-through in collection tube.

2.5 Transfer the remaining lysate to the above RNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 12,000 ×g for 30 seconds. Discard the flow-through in collection tube.

2.6 According to the ratio of 20 µL **DNase I Magic Buffer** and 10 µL **DNase I** (3 U/µL) per sample, mix **DNase I Magic Buffer** and **DNase I** (3 U/µL) by pipetting up and down to prepare sufficient DNase I working solution.

2.7 Apply 30 µL DNase I working solution to the center of membrane. Incubate at room temperature for 15 min.

Note:

- *Don't touch the membrane.*
- *The DNase I working solution should be prepared just prior to use.*

2.8 Add 600 µL **Wash Buffer A** to RNA Spin Column, centrifuge at 12,000 ×g for 30 seconds. Discard the flow-through in collection tube.

2.9 Add 600 µL **Wash Buffer A** to RNA Spin Column, centrifuge at 12,000 ×g for 30 seconds. Discard the flow-through in collection tube.

2.10 Add 600 µL **Wash Buffer B** to RNA Spin Column, centrifuge at 12,000 ×g for 30 seconds. Discard the flow-through in collection tube.

2.11 Add 600 µL **Wash Buffer B** to RNA Spin Column, centrifuge at 12,000 ×g for 30 seconds. Discard the collection tube with flow-through.

2.12 Place the RNA Spin Column in a clean 2 mL collection tube, centrifuge at 12,000 ×g for 3 min. Discard the collection tube with flow-through.

2.13 Place the RNA 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.

2.14 According to the ratio of 100 µL **Buffer RTE** and 5 µL **RNA Protection Buffer** per sample, mix **Buffer RTE** and **RNA Protection Buffer** by pipetting up and down to prepare sufficient **Buffer RTE Mix.**

2.15 Apply 80~100 µL **Buffer RTE Mix** to the center of the membrane. Do not touch the membrane. Incubate at 56℃ for 3 min. Centrifuge at 12,000 ×g for 1 min.

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

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

Performance Characteristics

The extraction efficacy of the kit was established by testing of six clinical tissue samples.

- Extracted RNA: Mean A260 ≥ 0.25, and Mean A260/A280 ratio ≥ 1.6.

Limitations

1) The quality of extracted RNA 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 RNA.

   The existence of RNase in laboratory environment may lead to degradation of the extracted RNA, please remove RNase of all the equipment and consumables before DNA or RNA extraction.

General Notes

If, during the use of this device or as a result of its use, a serious incident has occurred, 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
	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.
V02	2025-02-14	Update European and Swiss Authorized Representative