# AmoyDx® Tissue RNA Kit

# (Spin Column)

**For purification of RNA from human tissue or pleural effusion precipitation**

Instruction for Use

For Research Use Only

REF 8.02.24601X036G 36 tests

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## 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.

## 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 | Quantity |
|---|---|---|
| — | **RNA Spin Columns** | 36 tubes |
| — | **Collection Tubes (2 mL)** | 72 tubes |
| — | **Centrifugal Tubes (1.5 mL)** | 54 tubes/bag ×2 |
| 1 | **Buffer RLB** | 31 mL/vial ×1 |
| 2 | **Proteinase K Solution** | 900 µL/tube ×1 |
| 3 | **DNase I Magic Buffer** | 1.5 mL/tube ×1 |
| 4 | **DNase I (30 U/µL)** | 40 µL/tube ×1 |
| 5 | **RNase-free Water** | 1.5 mL/tube ×1 |
| 6 | **Wash Buffer A (concentrate)** | 13 mL/vial ×2 |
| 7 | **Wash Buffer B (concentrate)** | 6 mL/vial ×2 |
| 8 | **RNA Protection Buffer** | 200 µL/tube ×1 |
| 9 | **Buffer RTE** | 1.5 mL/tube ×3 |
| 10 | **Buffer TC** | 15 mL/vial ×1 |

***Note:***

1) ***Buffer RLB*** *contains 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 (concentrate)*** *and* ***Wash Buffer B (concentrate)****, 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 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

### 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*** contains 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.
- 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 time 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 (concentrate)** and **Wash Buffer B (concentrate)**, 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℃.

## 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.

## Symbols

| | | | |
|---|---|---|---|
|  | Manufacturer |  | Catalogue Number |
|  | Batch Code |  | Use By |
|  | Contains Sufficient for <n> Tests |  | Temperature Limitation |
|  | Consult Instructions For Use |  | Keep Dry |
|  | This Way Up |  | Fragile, Handle With Care |