AmoyDx
艾德生物

CE IVD

AmoyDx® FFPE RNA Kit

(Spin Column)

Instructions for Use

REF 8.02.0003 36 tests/kit

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This importer information is only applicable for EU market

Version: V02

Intended Use

The AmoyDx® FFPE RNA Kit is specially designed for isolation and purification of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. The purified RNA is suitable for downstream applications such as reverse transcription, RT-PCR, and real-time quantitative RT-PCR (qRT-PCR).

Intended User

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

Principle

FFPE specimen tissue sections are first deparaffinated with xylene/ethanol method, then incubated in buffer RTL and Proteinase K solution, to release RNA from the sections. A short incubation at a higher temperature partially reverses formalin crosslinking of the released nucleic acids, improving RNA yield and quality as well as RNA performance in downstream enzymatic assays. Next, genomic DNA in the solution is removed with the DNase I. The lysate is mixed with Buffer RPB and ethanol to provide appropriate binding conditions for RNA, and the sample is then applied to a RNA spin column, where the total RNA binds to the membrane and impurities are removed with wash buffer. The total 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 RTL**	Buffer RTL 裂解液 RTL	10 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	**Buffer RPB**	Buffer RPB 结合液 RPB	15 mL ×1

6	Wash Buffer A	Wash Buffer A 洗涤液 A	13 mL ×1
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 RTE	1.5 mL × 3
10	RNase-free Water	RNase-free Water 无核酸酶水	1.5 mL ×1
11	Tissue Tracer	Tissue Tracer 沉淀剂	200 µL ×1

Note:

1) ***Buffer RPB*** *and* ***Wash Buffer A*** *contain a guanidine salt, not compatible with disinfectants containing bleach.*
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.*
3) *For the first time use, add 360 µL* ***RNase-free Water*** *into* ***DNase I (30 U/µL)*** *to obtain* ***DNase I (3 U/µL)*** *solution, mix well by pipetting gently up and down.*

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) Xylene.
3) Microcentrifuge (13000×g adjustable).
4) Vortexer.
5) Palm centrifuge.
6) Thermomixer with block for 1.5 mL tube (37~80℃ adjustable and 500 rpm adjustable).
7) Adjustable pipettors and nuclease-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 RPB** 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 nuclease-free filtered pipette tips when handling samples and reagents to prevent the RNase contamination and 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

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation of nucleic acids. To limit the extent of RNA fragmentation, be sure to:

1) Fix tissue samples in at least 10 times volume of 10% neutral buffered formalin solution as quickly as possible after surgical removal.
2) The tissue thickness, volume of neutral buffered formalin and duration time will affect the quality of tissue fixation. Insufficient fixation or over-fixation may lead to poor performance in downstream assays. For surgical tissue specimens, the fixation time should be less than 24 hours. For biopsy specimens, the fixation time should be less than 12 hours.
3) Use low-melting paraffin for embedding, as high-melting paraffin may cause nucleic acid fragmentation.
4) Store FFPE tissue specimens at 2~8℃, as long-term storage at high temperature (e.g. room temperature) may increase the nucleic acid degradation and fragmentation.
5) The FFPET slide preparation process may cause nucleic acid loss. It's recommended to cut FFPET block to FFPET scrolls directly in centrifuge tube for RNA extraction.
6) If use FFPET slide for RNA extraction, it's recommended to add a drop of xylene on the top of the slide to dissolve the remaining FFPE tissue after the scraping and pipet the solution into the centrifuge tube.
7) The storage time of FFPE tissue sample should be less than 3 years.
8) The FFPET sample requirement for RNA extraction differ according to the FFPE tissue type (Table 2). The FFPET sample amounts directly affect the RNA yield.

Table 2 Recommended FFPET sections for RNA extraction

Tissue Type	Thickness	Amount
Surgical FFPET scrolls	5~8 μm (5 μm is recommended)	4~6 scrolls (5 scrolls are recommended)
Biopsy FFPET scrolls	5~8 μm (5 μm is recommended)	8~12 scrolls (10 scrolls are recommended)
FFPET slides	/	6~8 slides (7 slides are recommended)

Guidelines for sectioning paraffin blocks

Any method could be used for sectioning the paraffin blocks. General guidelines for sectioning paraffin blocks are outlined below:

1) Avoid nuclease contamination by using a clean, sharp blade and tweezers.
2) When multiple samples are processed, it's recommended to use the separate blade and tweezers for each sample to prevent the

cross-contamination. If not, place the blades and tweezers in xylene or terebenthene for 15 min for 2 times, and immerse in ethanol for 1 min then allow them to dry.

3) Timely clean the residual paraffin on the blade with 75% ethanol solution when cut multiple samples.

4) Wear disposable latex or nitrile gloves.

Assay Procedure

1. Deparaffination

1.1 Take sufficient FFPE tissue sections (see Table 2) in a 1.5 mL centrifugal tube.

1.2 Add 1 mL xylene and 2 µL Tissue Tracer, close the lid and vortex the tube vigorously for 10 seconds. Incubate at 56℃ for 3 min and mix by vortexing for 10 seconds. Centrifuge at 13000×g for 2 min at room temperature. Remove the supernatant by pipetting from top to down.

Note:

- *Do not touch the precipitate.*
- *If deparaffinization is not complete, repeat step 1.2.*

1.3 Add 1 mL ethanol (96~100%) and 2 µL Tissue Tracer to the precipitate, and vortex the tube for 10 seconds to remove the paraffin from tissue. Centrifuge at 13000×g for 2 min at room temperature. Remove the supernatant by pipetting from top to down. (Do not touch the precipitate).

1.4 Keep the tube open and allow the precipitate to dry at 56 °C for 1~10 min until the tissue show matt surface.

Note: *make sure all the residual ethanol has evaporated completely, as the ethanol may affect the RNA extraction.*

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.*
- *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 solution. If the reagents contain a precipitate, dissolved by heating at 50℃.*

2.1 Add 160 µL **Buffer RTL** and 20 µL **Proteinase K Solution** into the above centrifugal tube, and vortex to mix the solution well, briefly centrifuge for 5 seconds. Incubate at 56℃ for 30 min at 500 rpm in thermomixer.

2.2 Transfer the centrifugal tube to thermomixer and incubate at 80℃ for 30 min at 500 rpm.

2.3 Allow the sample to cool to room temperature, then briefly centrifuge for 5 seconds.

2.4 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.

Note: *the DNase I working solution should be prepared just prior to use.*

2.5 Add 30 μL DNase I working solution to the sample, mix gently by pipetting up and down. Incubate at 37℃ for 15 min.

2.6 Centrifuge at 13000×g for 3 min, transfer the supernatant into a new 1.5 mL centrifuge tube.

2.7 Add 340 μL **Buffer RPB** and 750 μL ethanol (96~100%), mix thoroughly by vortexing. Briefly centrifuge for 5 seconds.

2.8 Transfer 650 μL lysate in the centrifugal tube to the RNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 13000×g for 30 seconds. Discard the flow-through in collection tube.

2.9 Transfer the remaining lysate to the RNA Spin Column without wetting the rim, close the lid and centrifuge at 13000×g for 30 second. Discard the flow-through in collection tube.

Note: *the entire lysate is about 1300 μL, which is divided into two times for spin.*

2.10 Add 600 μL **Wash Buffer A** to RNA Spin Column, centrifuge at 13000×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 13000×g for 30 seconds. Discard the flow-through in collection tube.

2.12 Add 600 μL **Wash Buffer B** to RNA Spin Column, centrifuge at 13000×g for 30 seconds. Discard the collection tube with the flow-through.

2.13 Place the RNA Spin Column in a new 2 mL collection tube, centrifuge at 13000×g for 3 min. Discard the collection tube.

2.14 Place the RNA Spin Column in a new 1.5 mL centrifugal tube. Keep the RNA Spin Column open and incubate at 56℃ for 3 min.

2.15 According to the ratio of 100 μL **Buffer RTE** and 5 μL **RNA Protection Buffer**, mix **Buffer RTE** and **RNA Protection Buffer** by pipetting upand down to prepare sufficient **Buffer RTE Mix.**

Note: *the Buffer RTE Mix should be prepared just prior to use.*

2.16 Apply 80~100 μL **Buffer RTE** Mix to the center of the membrane (Do not touch the membrane). Close the lid and incubate at 56℃ for 2 min. Centrifuge at 13000×g for 1 min.

Note: *Two times elution makes for higher RNA yield. (eg. 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 13,000×g for 1 min. Then apply another 50μL Buffer RTE Mix to the center of the membrane, incubate at 56℃ for 2 min and centrifuge at 13,000×g for 1 min.)*

2.17 The eluted RNA in the centrifugal tube is ready for use immediately. If the RNA is not used within 2 hours, it should be stored at -70℃.

Performance Characteristics

The extraction efficacy of the kit was established by testing of six clinical FFPE 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, formalin fixation, paraffin embedding and storage conditions.
2. Sample quality has a high impact on quality and amount of the purified RNA.
3. Due to fixation and embedding conditions, nucleic acids in FFPE tissue samples are usually heavily fragmented and chemically modified by formaldehyde. The extracted RNA from FFPE tissue should not be used in downstream applications that require full-length RNA.

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
	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