AmoyDx
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AmoyDx® FFPE DNA/RNA Kit

(Spin Column)

For purification of DNA and RNA from formalin-fixed, paraffin-embedded tissue sections

Instructions for Use

REF 8.02.0002 36 tests/kit

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This importer information is only applicable for EU market

Version: V02

Intended Use

The AmoyDx® FFPE DNA/RNA Kit provides silica-based membrane and special lysis buffer system for FFPE tissue DNA/RNA extraction effectively. This kit is specially designed for simultaneously isolation and purification of DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. The purified DNA/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 DNA/RNA Kit is intended to be used by laboratory professionals only.

Principle

FFPE specimen tissue sections are first deparaffinized with xylene/ethanol method, then incubated in Buffer RTL and Proteinase K solution, to release DNA and RNA from the sections. After centrifugation RNA is in the supernatant, and DNA is in underlying the precipitates.

DNA Extraction: The precipitates incubated in Buffer DTL and Proteinase K solution to completely release DNA. A short incubation in Buffer DES at a higher temperature partially reverses formalin crosslinking of the released nucleic acids, improving DNA yield and quality as well as DNA performance in downstream assays. The lysate is mixed with Buffer DTB and ethanol to provide appropriate binding conditions for DNA, then the mixture is applied to a DNA spin column, where the DNA binds to the membrane and impurities are removed with wash buffer. The DNA is eluted in Buffer DTE.

RNA Extraction: The supernatant incubated at 80℃ to partially reverse 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 RTB 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
—	**DNA Spin Columns**	**DNA Spin Columns** **DNA 吸附柱**	36 pcs ×1
—	**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 ×2
—	**Centrifugal Tubes (1.5 mL)**	**Centrifugal Tubes (1.5 mL)** **1.5 mL 离心管**	72 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	1.8 mL ×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 RTB	Buffer RTB 结合液 RTB	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	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
1	Buffer DTL	Buffer DTL 裂解液 DTL	10 mL ×1
2	Buffer DES	Buffer DES 修复液 DES	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
7	RNase A (100 mg/mL)	RNase A RNA 消化酶	90 μL ×1

Note:

1) ***Buffer RTB***, ***Wash Buffer A***, ***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* ***Wash Buffer A****, add 24 mL ethanol (96~100%) into* ***Wash Buffer B****, add 17 mL ethanol (96~100%) into* ***Buffer DW1****, and add 24 mL ethanol (96~100%) into* ***Buffer DW2.*** *Mix all the solutions thoroughly. Tick the check box on the bottle label.*

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. 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. Xylene.
3. Thermomixer with block for 1.5 mL tube (37~90℃ adjustable and 500 rpm adjustable).
4. Microcentrifuge (13,000 ×g adjustable).
5. Vortexer.
6. Palm centrifuge.
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. 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 RTB**, **Wash Buffer A**, **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.
- Use separate, dedicated pipettes and nuclease-free filtered pipette tips when handling samples and reagents to prevent the nuclease 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 DNA/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 FFPE tissue slide preparation process may cause nucleic acid loss. It's recommended to cut FFPE tissue block to FFPE tissue

scrolls directly in centrifuge tube for DNA/RNA extraction.

6) If use FFPE tissue slide for DNA/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 FFPE tissue sample requirement for DNA/RNA extraction differ according to the FFPE tissue type (Table 2). The FFPE tissue sample amounts directly affect the DNA/RNA yield.

Table 2 Recommended FFPE tissue sections for DNA/RNA extraction

Tissue Type	Thickness	Amount
Surgical FFPE tissue scrolls	5~8 μm (5 μm is recommended)	4~6 scrolls (5 scrolls are recommended)
Biopsy FFPE tissue scrolls	5~8 μm (5 μm is recommended)	8~12 scrolls (10 scrolls are recommended)
FFPE tissue 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. Deparaffinization

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 13,000 ×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 13,000 ×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. Tissue Lysis

2.1 Add 200 µL **Buffer RTL** and 25 µL **Proteinase K Solution** into the tube above, mix gently by vortexing. Briefly centrifuge the solution and incubate at 56℃ for 30 min at 500 rpm in the thermomixer.

2.2 Centrifuge at 13,000 ×g for 2 min. Transfer 180 µL supernatant to a clean 1.5 mL centrifugal tube for RNA extraction.

2.3 The remaining solution and precipitate is for DNA extraction. Add 140 µL **Buffer DTL** and 15 µL **Proteinase K Solution** into the tube, mix by vortexing. Briefly centrifuge for 5 seconds and incubate at 56℃ for 1 hour at 500 rpm in the thermomixer.

2.4 Perform RNA extraction during DNA incubation.

3. RNA Extraction

Note:

- *For the first time use, please add 17 mL ethanol (96~100%) into **Wash Buffer A**, add 24 mL ethanol (96~100%) into **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) to obtain 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℃.*
- *It's recommended to conduct RNA extraction in biosafety cabinet to prevent RNA degradation by RNase in laboratory environment.*

3.1 Adjust the temperature of thermomixer as 80℃, incubate the centrifugal tube containing 180 µL supernatant at 80℃ for 30 min at 500 rpm.

3.2 Take out the centrifuge tube from the thermomixer and cool to room temperature, then briefly centrifuge for 5 seconds.

3.3 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 get DNase I working solution.

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

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

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

3.6 Transfer 650 µL lysate to the RNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 13,000 ×g for 30 seconds. Discard the flow-through in collection tube.

3.7 Transfer the remaining solution to the RNA Spin Column, without wetting the rim, close the lid and centrifuge at 13,000 ×g for 30 seconds. Discard the flow-through in collection tube.

3.8 Add 600 µL **Wash Buffer A** to RNA Spin Column, centrifuge at 13,000 ×g for 30 seconds. Discard the flow-through in collection tube.

3.9 Add 600 µL **Wash Buffer B** to RNA Spin Column, centrifuge at 13,000 ×g for 30 seconds. Discard the flow-through in collection tube.

3.10 Add 600 µL **Wash Buffer B** to RNA Spin Column, centrifuge at 13,000 ×g for 30 seconds. Discard collection tube with flow-through.

3.11 Place the RNA Spin Column in a clean 2 mL collection tube, centrifuge at 13000 ×g for 3 min. Discard the collection tube with flow-through.

3.12 Place the RNA Spin Column in a clean 1.5 mL centrifugal tube. Open the tube and incubate at 56℃ for 3 min.

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

3.14 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 13,000 ×g for 1 min.

Note:

- *The Buffer RTE Mix should be prepared prior to use.*
- *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 membrane, incubate at 56 ℃ for 2 min and centrifuge at 13,000 ×g for 1 min. Then apply another 50 µL Buffer RTE Mix into membrane, incubate at 56℃ for 2 min and centrifuge at 13,000 ×g for 1 min.*)

3.15 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℃.

4. 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**, mix each of them thoroughly, 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℃.*

4.1 Take out the tube from the thermomixer, briefly centrifuge for 5 seconds.

4.2 Add 10 μL **Buffer DES**, mix gently by vortexing and briefly centrifuge for 5 seconds. Then incubate at 90℃ for 1 hour at 500 rpm in the thermomixer.

4.3 Take out the centrifuge tube from the thermomixer, briefly centrifuge for 5 seconds.

Note: *If RNA-free genomic DNA is required, allow the sample to cool to room temperature, add 2 μL* ***RNase A (100 mg/mL)*** *and incubate at room temperature for 5 min.*

4.4 Successively add 350 μL **ethanol** (96~100%) and 150 μL **Buffer DTB**, mix by vortexing and briefly centrifuge for 5 seconds.

4.5 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 13,000 ×g for 30 seconds. Discard the flow-through in collection tube.

4.6 Add 600 μL **Buffer DW1** to DNA Spin Column, centrifuge at 13,000 ×g for 30 seconds. Discard the flow-through in collection tube.

4.7 Add 600 μL **Buffer DW2** to DNA Spin Column, centrifuge at 13,000 ×g for 30 seconds. Discard the collection tube with flow-through.

4.8 Place the DNA Spin Column in a clean 2 mL collection tube, centrifuge at 13,000 ×g for 3 min. Discard the collection tube with flow-through.

4.9 Place the DNA Spin Column in a clean 1.5 mL centrifugal tube. Keep the tube open to dry at 56℃ for 3 min.

4.10 Apply 30~100 μL **Buffer DTE** to the center of the membrane. Do not touch the membrane. Incubate at room temperature for 2 min. Centrifuge at 13,000 ×g for 1 min.

Note: *Two times elution makes for higher DNA yield. (e.g. If elution volume is 100 μL, firstly apply 50 μL Buffer DTE to the center of membrane, incubate for 2 min at 56℃ and centrifuge at 13,000 ×g for 1 min. Then apply another Buffer DTE to the center of membrane, incubate for 2 min at 56℃ and centrifuge at 13,000 ×g for 1 min)*

4.11 The eluted DNA in the centrifugal tube is ready for use immediately. If DNA is not used within 6 hours, it should be stored at -20℃.

Performance Characteristics

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

- Extracted DNA: Mean A260 ≥ 0.2, and Mean A260/A280 ratio ≥ 1.6.
- Extracted RNA: Mean A260 ≥ 0.25, and Mean A260/A280 ratio ≥ 1.6.

Limitations

1) The quality of extracted DNA and 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 DNA/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 DNA/RNA from FFPE tissue should not be used in downstream applications that require full-length DNA/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
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.
V02	2025-02-14	Update European and Swiss Authorized Representative