AmoyDx® FFPE DNA Kit

(Spin Column)

Instructions for Use

REF 8.02.0001 36 tests/kit

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This importer information is only applicable for EU market

Version: V02

Intended Use

The AmoyDx® FFPE DNA Kit is specially designed for isolation and purification of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. The purified DNA is suitable for downstream applications such as real-time PCR and Sequencing.

Intended User

The AmoyDx® FFPE DNA 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 DTL and Proteinase K solution, to release DNA from the sections. 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.

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 DES**	**Buffer DES** **修复液 DES**	800 µL ×1
4	**Buffer DTB**	**Buffer DTB** **结合液 DTB**	10 mL ×1
5	**Buffer DW1**	**Buffer DW1** **洗涤液 DW1**	13 mL×1
6	**Buffer DW2**	**Buffer DW2** **洗涤液 DW2**	6 mL ×1
7	**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) Xylene.
3) Microcentrifuge.
4) Vortexer.
5) Palm centrifuge.
6) Water bath or heated orbital incubator (37~90℃ adjustable).
7) Sterile, Nuclease-free pipet tips.
8) Recommend: microtome suitable for sectioning paraffin-embedded tissue that is capable of producing 5~10 μm sections.

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 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

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

1) Fix tissue samples in 4~10% neutral formalin solution as quickly as possible after surgical removal.
2) Use a fixation time of 14~24 hours (longer fixation time leads to more severe DNA fragmentation, resulting in poor performance in downstream assays).
3) Thoroughly dehydrate samples prior to embedding (residual formalin maybe inhibit the digestion of the Proteinase K).
4) The starting material for DNA purification should be freshly cut sections of FFPE tissue, each with a thickness of less than 10 μm. (Thicker sections may result in lower DNA yields, even after prolonged incubation with proteinase K).

5) The FFPE tissue area should be 0.5~1 cm^2. If the FFPE tissue surface area is less than 0.5 cm^2, please use more sections.

6) The storage time of FFPE sample should be less than 3 years.

Guidelines for Sectioning Paraffin Blocks

To use this kit, it needs 5~10 μm sections of the tissue in paraffin block. You may use any method for sectioning the paraffin blocks. General guidelines for sectioning paraffin blocks are outlined below:

1) Avoid nuclease contamination by using a clean, sharp microtome blade and tweezers.

2) When multiple samples are processed, clean the microtome blade and tweezers with DNase-inactivating agents to avoid cross-contamination of nucleic acids and DNases. UV irradiation for 10 minutes is recommended after cleaning.

3) Always wear latex or vinyl gloves.

4) Cut 5~10 μm thick sections from trimmed paraffin blocks with a tissue surface area about 0.5~1 cm^2.

Assay Procedure

1. Deparaffinization

1.1 Using a scalpel, trim excess paraffin off the sample block.

1.2 Cut sections with a thickness of 5~10 μm and a surface area between 0.5~1 cm^2.

1.3 Immediately place 2~5 sections in a 1.5 mL centrifugal tube.

1.4 Add 1 mL xylene, close the lid and vortex vigorously for 10 seconds.

1.5 Centrifuge at 13000×g for 2 min at room temperature.

1.6 Remove the supernatant by pipetting (do not remove any of the pellet).

1.7 Add 1mL ethanol (96~100%) to the pellet, and mix by vortexing for 10 seconds.

1.8 Centrifuge at 13000×g for 2 min at room temperature.

1.9 Remove the supernatant by pipetting (do not remove any of the pellet).

1.10 Open the tube and incubate at room temperature for 10 min, or at 37℃ for 5 min. Make sure all residual ethanol has evaporated before proceeding.

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 180 μL **Buffer DTL** and 20 μL **Proteinase K Solution**, mix by vortexing.

2.2 Incubate at 56℃ for 1 hour for lyse the sample tissue. If the tissue has not been completely lysed, or need higher concentration

of DNA, incubate for further time or overnight.

2.3 Add 10 µL **Buffer DES**. Transfer the centrifugal tube to heated orbital incubator and incubate at 90℃ for 1 hour.

2.4 Briefly centrifuge for 5~10 seconds. 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 for 5 min at room temperature.

2.5 Add 200 µL **Buffer DTB** and 200 µL ethanol (96~100%), mix by vortexing.

2.6 Briefly centrifuge for 5~10 seconds.

2.7 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 10000×g for 1 min.

2.8 Discard the flow-through in collection tube.

2.9 Add 600 µL **Buffer DW1** to DNA Spin Column, centrifuge at 10000×g for 1 min.

2.10 Discard the flow-through in collection tube.

2.11 Add 600 µL **Buffer DW2** to DNA Spin Column, centrifuge at 10000×g for 1 min.

2.12 Discard the collection tube with flow-through.

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

2.14 Discard the collection tube with flow-through.

2.15 Place the DNA Spin Column in a clean 1.5 mL centrifugal tube.

2.16 Apply 30~100 µL **Buffer DTE** to the center of the membrane. Do not touch the membrane.

2.17 Incubate at room temperature for 1~5 min.

2.18 Centrifuge at 13000×g for 1 min.

2.19 The eluted DNA is immediately ready for use or for storage under -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 FFPE tissue 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, formalin fixation, paraffin embedding and storage conditions.

2) Sample quality has a high impact on quality and amount of the purified DNA.

3) Due to fixation and embedding conditions, nucleic acids in FFPE samples are usually heavily fragmented and chemically modified by formaldehyde. The extracted DNA from FFPE tissue should not be used in downstream applications that require full-length 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.
V02	2025-02-14	Update European and Swiss Authorized Representative