AmoyDx
艾德生物

CE IVD

AmoyDx® Magnetic FFPE DNA Extraction Kit

Instructions for Use

REF 8.02.0098 36 tests/kit

Amoy Diagnostics Co., Ltd.
No. 39, Dingshan Road, Haicang District,
361027 Xiamen, P. R. China
Tel: +86 592 6806835
Fax: +86 592 6806839
E-mail: sales@amoydx.com
Website: www.amoydx.com

EC REP

Qarad EC-REP BV
Pas 257,
2440 Geel, Belgium

Umedwings Netherlands B.V.
Treubstraat 1, 2288EG, Rijswijk, the Netherlands
SRN: NL-IM-000000454
This importer information is only applicable for EU market

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Intended Use

The AmoyDx® Magnetic FFPE DNA Extraction Kit is specially designed for isolation, enrichment 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® Magnetic FFPE DNA Extraction Kit is intended to be used by laboratory professionals only.

Principle

The paraffin on FFPE specimen tissue need to be firstly removed with deparaffinization solution, then, with a high-efficiency lysis buffer system, combined with nano-magnetic beads adsorption technology, DNA will be extracted from samples efficiently.

Kit Contents

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

Table 1 Kit Contents

Tube No.	Component	Symbol	Quantity
—	Centrifugal Tubes (1.5 mL)	Centrifugal Tubes (1.5 mL) 1.5 mL 离心管	72 pcs ×1
1	Tissue Tracer	Tissue Tracer 沉淀剂	200 µL ×1
2	Lysis Buffer DL	Lysis Buffer DL 裂解液 DL	12 mL ×1
3	Proteinase K Solution	Proteinase K Solution 蛋白酶 K 溶液	1.3 mL ×1
4	Buffer DES	Buffer DES 修复液 DES	3 mL ×1
5	RNase A Solution (10 mg/mL)	RNase A Solution RNase A 溶液	450 µL ×1
6	Binding Buffer DB	Binding Buffer DB 结合液 DB	14 mL ×1
7	Magnetic Beads M	Magnetic Beads M 磁珠 M	1.6 mL ×1
8	Wash Buffer D1	Wash Buffer D1 洗涤液 D1	13 mL ×1
9	Wash Buffer D2	Wash Buffer D2 洗涤液 D2	6 mL ×2
10	Elution Buffer DE	Elution Buffer DE 洗脱液 DE	5 mL ×1

Note:

1) ***Binding Buffer DB*** *and* ***Wash Buffer D1*** *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 D1*** *and mix thoroughly; add 24 mL ethanol (96~100%) into* ***Wash Buffer D2*** *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 stored dry at room temperature (10~30℃).

Materials Required but Not Supplied

1) Deparaffinization solution. (Xylene or commercial deparaffinization solution)

2) Ethanol (96~100%)
3) Isopropyl alcohol
4) Water bath or heated orbital incubator (37~90℃ adjustable)
5) 1.5 mL Magnetic stand
6) Microcentrifuge (13000×g adjustable)
7) Vortexer
8) Palm centrifuge
9) Sterile, Nuclease-free pipet tips
10) 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

- **Binding Buffer DB** and **Wash Buffer D1** 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 cross-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) Immediately place the sample in no less than 10 times the volume of 10% neutral formalin solution for fixation after surgical removal.
2) The recommended fixation time for surgical sample is under 24 hours, for FNA sample is under 12 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.
   For surgical sample, the thickness should be 5~8 μm. (5 μm is recommended) And 4~6 sections (5 is recommended) are needed.
   For FNA sample, the thickness should be 5~8 μm. (5 μm is recommended) And 8~12 sections (10 is recommended) are needed.
   Thicker sections may result in lower DNA yields, even after prolonged incubation with Proteinase K.
5) The storage time of FFPE sample should be less than 3 years.

Guidelines for Sectioning Paraffin Blocks

To use this kit, it needs 5~8 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) 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 Prepare the starting material according to the requirements in "**Specimen Collection, Transport and Storage**" part.
1.2 Place the starting material into a 1.5 mL centrifugal tube.
1.3 Add 1 mL deparaffinization solution and 5 μL **Tissue Tracer**, close the lid and vortex vigorously for 10 seconds.
1.4 Briefly centrifuge for 5~10 seconds.
1.5 Incubate at 56℃for 3 min. (Shake at 500 rpm if available)
1.6 Mix by vortexing for 10 seconds.
1.7 Centrifuge at 13000×g for 2 min.
1.8 Remove the supernatant by pipetting (do not remove any of the pellet).
1.9 Briefly centrifuge for 5~10 seconds and remove the rest of supernatant with smaller pipet tip (do not remove any of the pellet).

2. DNA Extraction

2.1 Add 285 μL **Lysis Buffer DL** and 30 μL **Proteinase K Solution**, mix by vortexing and then briefly centrifuge.
2.2 Incubate at 56℃for 1 hour for lysing the sample tissue (Shake at 500 rpm if available). If the tissue has not been completely lysed, or need higher concentration of DNA, incubate for longer time or overnight.
2.3 Add 75 μL **Buffer DES,** mix by vortexing and then briefly centrifuge.
2.4 Incubate at 90℃ for 1 hour (Shake at 500 rpm if available) and then briefly centrifuge.
2.5 Allow the sample to cool to room temperature, add 10 μL **RNase A Solution (10 mg/mL)** and incubate for 5 min at room temperature.

2.6 Add 550 µL isopropyl alcohol, 350 µL **Binding Buffer DB**, and 40 µL **Magnetic Beads M** in order, mix by inverting 20 times .

Note:

- *The **Magnetic Beads M** need to be well mixed by vortexing 1 min prior use.*

2.7 Stand at room temperature for 3 minutes.

2.8 Mix by inverting 20 times again, then stand at room temperature for 3 min.

2.9 Briefly centrifuge and then place the centrifuge tubes onto the magnetic stand for 3 min until the solution turns clear. Gently remove and discard the supernatant while the centrifuge tube is on the magnetic stand. **Do not** touch the beads with pipette tip.

2.10 Add 800 µL **Wash Buffer D1**, mix by vortexing for 20 sec.

Note:

- *For the first time use, please add 17 mL ethanol (96~100%) into **Wash Buffer D1** and tick the check box on the bottle label.*

2.11 Briefly centrifuge and then place the centrifuge tubes onto the magnetic stand for 1 min until the solution turns clear. Gently remove and discard the supernatant while the centrifuge tube is on the magnetic stand. **Do not** touch the beads with pipette tip.

2.12 Add 800 µL **Wash Buffer D2**, mix by vortexing for 20 sec.

Note:

- *For the first time use, please add 24 mL ethanol (96~100%) into **Wash Buffer D2**, and tick the check box on the bottle label.*

2.13 Briefly centrifuge and then place the centrifuge tubes onto the magnetic stand for 1 min until the solution turns clear. Gently remove and discard the supernatant while the centrifuge tube is on the magnetic stand. **Do not** touch the beads with pipette tip.

2.14 Add 800 µL **Wash Buffer D2** again, mix by vortexing for 20 sec.

2.15 Briefly centrifuge and then place the centrifuge tubes onto the magnetic stand for 1 min until the solution turns clear. Gently remove and discard the supernatant while the centrifuge tube is on the magnetic stand. **Do not** touch the beads with pipette tip.

2.16 Briefly centrifuge and then place the centrifuge tubes onto the magnetic stand for 1 min, and remove the rest of the supernatant with smaller pipette tip.

2.17 Air dry the beads at 56℃ for 1~5 min with the lid opened until the surface is matt.

Note:

- ***Do not** over-dry the beads. This may result in lower recovery of DNA.*

2.18 Add 20~100 µL **Elution Buffer DE** mix by vortexing and then briefly centrifuge.

Note:

- *If the beads cannot completely resuspend by vortexing, re-mix them by gently pipetting.*

2.19 Briefly centrifuge and then incubate at 56℃ for 2 min.

2.20 Briefly centrifuge and then place the centrifuge tubes onto the magnetic stand for 3 min until the solution turns clear.

2.21 Gently transfer the supernatant to a clean nuclease-free 1.5 mL centrifuge tube without disturbing the bead pellet.

Note:

- *It is recommended to use the extracted DNA immediately, otherwise please store it at -20℃*

Performance Characteristics

The extraction efficacy of the kit was established by testing 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 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	!	Health hazard

Revision History

Revision	Effective Date	Revision History
V01	2023.02.13	First edition