One-Step FFPE & FNA DNA Purification Kit

Formalin fixation and paraffin embedding (FFPE) tissues and Fine Aspiration Biopsy (FNA) are popular sources of archival tissue samples that pathologists have used for decades. It enables long-term, room-temperature preservation of tissue samples, making it extremely handy. Because it has been the standard for so long, it is also the primary source of biological research materials. One of the critical steps in molecular oncology diagnostics is obtaining high-quality genomic DNA. Mutation analysis of FFPE- or FNA-derived DNA aids in the diagnosis of most solid tumors. Tests such as Epidermal Growth Factor Receptor (EGFR), Kristen Ras Gene (KRAS), Neuroblastoma Ras Gene (NRAS), and Next Generation Sequencing for significantly mutated genes hot spot detection using FFPE DNA have become essential in determining specific cancer treatment.

However, DNA extraction from these tissues is a challenge mainly owing to formalin fixation, which causes cross-linking between proteins and DNA and distinct DNA strands. As a result, pure DNA is frequently extensively damaged and fragmented. The degree of fragmentation is determined by tissue type, sample age, and specific fixation procedures. These characteristics can cause problems in downstream applications, many of which use PCR. As a result, DNA purification from FFPE tissues must successfully extract highly fragmented DNA and reverse cross-linking generated by formalin fixation.

To extract enough genomic DNA (gDNA) for downstream analysis, several commercially available nucleic acid extraction kits require a minimum of one 10µm slide of starting material. Because these blocks are acquired from human patients, the extraction kit is critical because it defines the size of the slide needed and the number of analyses performed on each FFPE block. Because some stored tissues are too tiny to meet this condition, this limitation lowers the number of feasible applications. Furthermore, using these kits for DNA extraction might be time-consuming, and the "Bind-Wash-Elute" procedure may cause significant DNA loss.

BcMag™ One-Step FFPE & FNA DNA Purification Kit is designed to extract total nucleic acids from 2µm - 5µm Formalin-Fixed, Paraffin-Embedded (FFPE) tissue or Fine Aspiration Biopsy samples efficiently and sequentially. The kit employs our unique magnetic beads to efficiently remove paraffin from FFPE tissue samples in an aqueous buffer while simultaneously rehydrating the tissue. The procedure eliminates flammable and odorous xylene or d-limonene and the time-consuming cleanup of organic solvent from frequently hardly visible tissue pellets commonly employed for deparaffinization. Furthermore, the kit is unique because its proprietary magnetic beads remove PCR inhibitors (Fig.1) from samples in a single step without needing DNA extraction. Therefore, it increases nucleic acid yields and avoids DNA loss caused by the time-consuming "bind-wash-elute" procedure used in traditional DNA purification techniques. Following sample lysis, the straightforward one-step purification technique is ideal for the simultaneous processing of >96 samples and produces pure DNA in less than 30 minutes. Purified genomic DNA has the highest integrity and can be used in various downstream applications such as qPCR, mutation screening, microarray analyses, sequencing, Southern blotting, and SNP analysis.

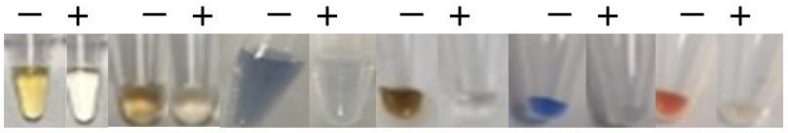

Fig.1 Cell lysate cleanup and PCR inhibitor removal

Workflow (Fig.2)

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Fig.2 Workflow of One-step FFPE and FNA DNA purification
1. To lyse the sample, add functional magnetic beads and proteinase K to the sample and incubate at 65°C.
2. Vortex/pipette the beads with the sample to capture the PCR inhibitors.
3. Separate the beads from the sample using a magnet.
4. Aspirate the supernatant containing the pure, ready-to-use DNA/RNA.

Features and Advantages:

- Rapid and efficient purification protocol: without prior DNA isolation for subsequent use in direct workflows, No liquid transfer, and One-tube.
- Ultrafast: Process 96 samples in less than an hour.
- Highest nucleic acids recovery rates: Minimal loss of DNA during extraction
- Effectively cell lysate cleanup and removes inhibitors: polyphenolic compounds, humic/fulvic acids, acidic polysaccharides, tannins, melanin, heparin, detergents, denim dyes, divalent cations such as Ca^{2+}, Mg^{2+}, etc.
- Highly improved active paraffin removal: No need for toxic organic solvents, such as xylene
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and organic reagents.
- High throughput: Compatible with many different automated liquid handling systems.

Handling and Storage: Store the kit components according to the table below on arrival.

Products

Components	Storage	50 preps, Cat # AJ-101	100 preps, Cat # AJ-102
BcMag™ U-DNA Beads	4°C	2.5 ml	5.0 ml
10x Lysis Buffer (100mM Tris-HCl, PH 9.0)	4°C	0.6 ml	1.2 ml
Proteinase K	-20°C	12.5 mg	25 mg
DTT(1M)	-20°C	15.4 mg	30.8 mg
Proteinase K Suspension Buffer	4°C	1.0 ml	2.0 ml

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

It's important to note that DNA isolated from FFPE samples has a lower molecular weight than DNA recovered from fresh or frozen samples. The degree of fragmentation is determined by the type and age of the sample, as well as the fixation conditions used. The kit is designed for the usage of FFPE mammalian tissue samples. It is not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue samples or FFPE samples obtained from nonmammalian tissues.

Notes

- DNA Yield: Varies (depends on sample size and type)
- DNA Size: Varies (depends on the quality of starting material
- Since there is no concentration step in the protocol, the concentration of the nucleic acid depends on the quality and quantity of the sample used.
- Quantification of the nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green. OD260 methods such as Nanodrop and UV-spectrophotometry are not-suitable.
- For long-term storage, store the extracted nucleic acids at -20°C.

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Materials Required by the User

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag™ Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag™ Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag™ Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag™ Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag™ 96-well Plate Magnetic Rack.	• BcMa™ 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible Racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the Time and speed should be optimized, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** ***IMPORTANT!*** If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	

A. Premix Beads solution Preparation

IMPORTANT!

1. Before pipetting, shake or Vortex the bottle to completely resuspend the Magnetic Beads.
2. Do not allow the magnetic beads to sit for more than 2 minutes before dispensing.
3. Proteinase K preparation: Provide protease K as lyophilized powder and dissolve at a 20 mg/ml concentration in Proteinase K Suspension Buffer. For example, 12.5 mg dissolved in 625 µl of Proteinase K Suspension Buffer. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded.
4. DTT solution preparation: Provide DTT as powder and dissolve at a concentration of 1M in ultrapure water. For example, 15.4 mg dissolved in 100µl ultrapure water. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.
5. Dilute DTT to a concentration of 10 mM from stock with ultrapure water and use it immediately. Discard unused DTT solution.
6. Prepare a fresh Master Mix following Table 2 for the number of samples to be processed, plus 10% more (e.g, if you have 10 samples, prepare Master Mix for 11). Add the following components to the reservoir.

Table 2. Premix Beads solution

Component	One well (100 µL reaction volume)
BcMag™ U-DNA Beads	50 µL
10x Lysis Buffer	10 µL
Proteinase K (20mg/ml)	12.5 µL
DTT (10 mM)	3 µL
Sample	x
ULTRAPURE WATER	x
Total	100 µL

B. Isolation procedure

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IMPORTANT!

- Pipet up and down premix beads solution in a reagent reservoir until the solution is homogeneous before dispensing.
- Do not allow the magnetic beads to sit for more than 5 minutes before dispensing.)

1. Transfer the sample to a new well of a 96well PCR plate. The size of the FFPE slides was 2µm - 5µm. Excess wax was removed from the slide before collecting FFPE mammalian tissue sections, and the tissue was collected with a scalpel and transferred to a 0.2ml PCR tube). For Fine Aspiration Biopsy **(**FNA**)** sample, use 5 µL-10 µLsample.

   Note: It is not designed for sample volumes larger than 2µm - 5µm. Only use sections that meet the size specification.
2. Transfer 100µL premix beads solution to the sample. (Note. Use 50 µl premix beads solution for <2.5 µm FFPE tissue section)
3. Mix the sample well by Vortex or pipetting.
4. Place the PCR plate/tube into a thermocycler and incubate at:
   a. 65°C for 60 minutes
   b. 90°C for 90 minutes
5. Remove the PCR plate/tube from the thermocycler and then mix the sample with beads by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes (see picture).

6. Centrifuge at 3500 rpm for 5 minutes.
7. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
8. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications. Using 1-5 ul in a 25µl for qPCR.

C. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA Recovery	• Poor starting sample material. • The sample was not completely lysed.	• Use better quality of the sample. • Add more samples. • Let the lysis proceed overnight at 65°C.
Ct value delays	Too many PCR inhibitors in the sample. Recovery DNA is so low.	1. Add 25-50 µL of BcMag™ U-DNA Beads to the extract solution and mix by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear. 2. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. Using 1-5 µL in a 25µl qPCR. The sample is ready for downstream applications.

Related Products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit**,** Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101

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One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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