ImaSpin® Genomic DNA Extraction Kit –FFPE

For extraction of genomic DNA from any type of FFPE sample.

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100 %)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

IDP buffer
IGT Buffer
IGB Buffer
IW1 Buffer
IW2 Buffer (Add Ethanol)
Elution Buffer
Proteinase K
PK Storage Buffer
IG Column
2 ml Collection Tubes

Storage and Stability:

1. This kit should be stored at room temperature.
2. Proteinase K should be stored at 4 °C upon arrival.

Description

ImaSpin® Genomic DNA Extraction Kit –FFPE is designed by patented technology for purification of total DNA (including genomic, mitochondrial and viral DNA) from Formalin-Fixed Paraffin-Embedded (FFPE). The protocol using a special non-toxic paraffin dissolving liquid with a convenient operation method to remove the paraffin in the sample, and using buffer contains chaotropic salt to lyse tissue and degrade protein. DNA will bind to special column. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer. The purified genomic DNA can be directly used for downstream applications, such as PCR, Real-time PCR, screening, southern blotting, STR analysis, LMD-PCR...etc.

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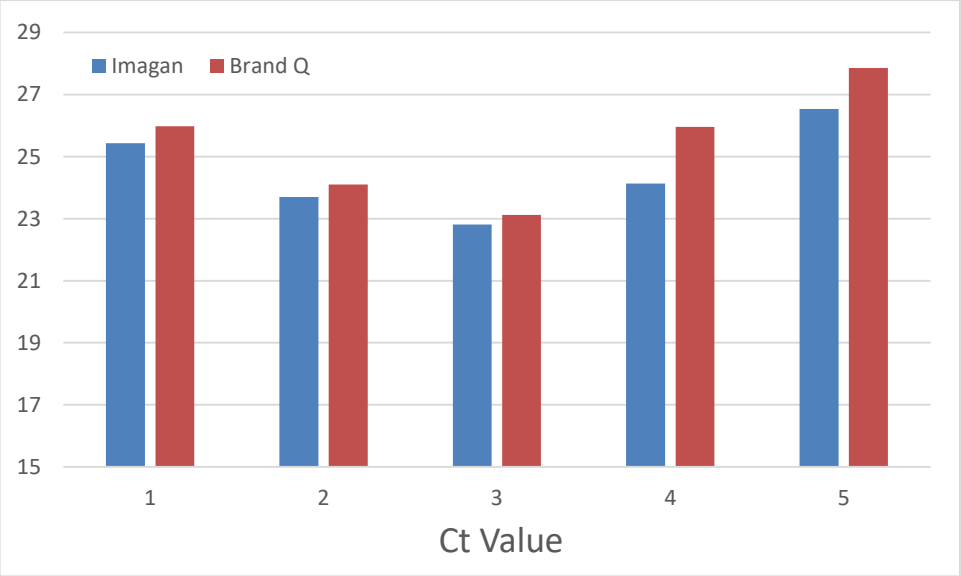

FFPE DNA was extracted using ImaSpin® Genomic DNA Kit - FFPE & Brand Q kit. Detected the concentration by real-time PCR, Ct value of the products extract by Imagen are lower than Brand Q.

Preparation before using

Add 1.1 ml PK Storage Buffer to the Proteinase K tube and mix by vortexing.
Store prepared Proteinase K (10 mg/ml) at 4 °C.

FFPE Protocol Procedure

1. Sample preparation.
   I. For Needle-Like FFPE Tissue Slices:
   Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube. Add 500 ul of IDP buffer, 400 ul of IGT Buffer and 20 of µl proteinase K, incubate at 56 °C for 1 hour or overnight until the sample has been completely lysed (base on tissue size).
   II. For Glass-Slide Samples:
   Drop several IDP buffer on the glass slide and scrape them from the slide carefully, then put in the bottom of to a 1.5 ml microcentrifuge tube. Add 500 ul of IDP buffer, 400 ul of IGT Buffer and 20 of µl proteinase K, incubate at 56 °C for 1 hour or overnight until the sample has been completely lysed (base on tissue size).

   Optional RNA Removal Step
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation, add 5 µl of RNase A (10 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 5 minutes.

2. Incubate at 90°C for 1 hour.
   NOTE: The 90°C incubation in IGT Buffer partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, please leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.
3. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
4. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes, and transfer the lower water layer liquid of the oil-water layer state to a new 1.5 ml microcentrifuge tube.
5. Add 400 ul of IGB Buffer then shake vigorously for 10 seconds, Incubate at 56 °C for 5 minutes.
   NOTE: Inverting the sample occasionally during incubation will facilitate Proteinase K digestion and cell lysis. Using an auto shaking system is more convenient when incubating samples.
6. Add 400 ul of absolute ethanol to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting.
   NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution.
7. Place an IG Column in a 2 ml Collection Tube.
8. Apply 700 µl of the mixture (including any precipitation) from previous step to the IG Column.
9. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes.
10. Discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
11. Apply the remaining mixture to the same IG Column and centrifuge a 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IG Column membrane, increase the centrifuge time until it passes completely.
12. Add 600 µl of IW1 Buffer to the IG Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
14. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IG Column.
15. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IG Column back in the 2 ml Collection Tube.
16. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
17. Transfer the dried IG Column to a clean 1.5 ml microcentrifuge tube.
18. Add 30 - 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix.

NOTE: If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.

19. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
20. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	➢ Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. ➢ Reduce the sample material. ➢ Following ethanol addition to the lysate, break up any precipitate as much as possible prior to loading to IG Column. ➢ Ensure Elution Buffer or water is added into the CENTER of the column matrix. ➢ Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	➢ Following the Wash Step, dry the IG column by incubate at 60 °C for 5 minutes. ➢ Use fresh blood, long term storage ample may result in fragmentation of genomic DNA. ➢ Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. ➢ If using water for elution, ensure the water pH is between 7.5 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA eluted in water should be stored at -20 °C to avoid degradation