ImaBeads® 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
ICGT Buffer
ICGL Buffer
ICGB Buffer
IBW1 Buffer
IW2 Buffer (Add Ethanol)
Elution Buffer
Proteinase K (Add PK Storage Buffer)
PK Storage Buffer
ImaBeads - 01

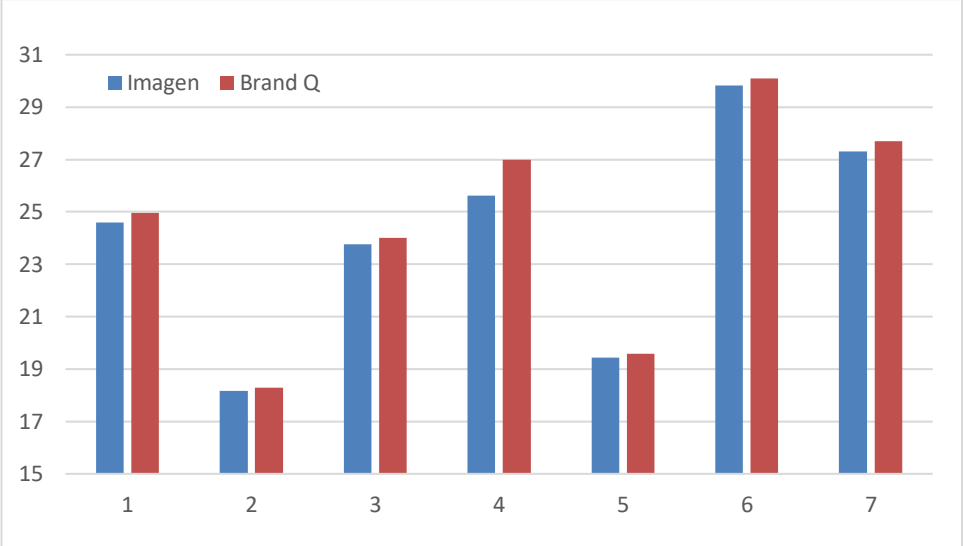
Storage and Stability:

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

Description

ImaBeads® 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 imabeads. 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.

ImaBeads® Genomic DNA Extraction Kit –FFPE



FFPE DNA was extracted using ImaBeads® 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, 500 ul of ICGT Buffer and 20 of µl proteinase K, incubate at 56 °C for 1 hour or 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, 500 ul of ICGT 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 ICGT 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. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes, and transfer 400µl of the lower water layer liquid of the oil-water layer state to a new 1.5 ml microcentrifuge tube.
4. Preheat required Elution Buffer (100 µl per sample) in 56°C (For DNA Elution Step).
5. Vortex **ImaBeads – 01** to ensure they are in suspension prior to initial use.
6. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
7. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
8. Add 100 µl of ICGL Buffer and 320 µl of ICGB Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting.
9. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube (prepared for use in step 7.) and mix with beads by vortexing for 10 minutes.
10. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
11. Add 800 µl of IBW1 Buffer and mix by vortexing for 1 minute.
12. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
13. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
14. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
15. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
16. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
17. Incubate the tube at 56 °C for 2 minutes to dry the ImaBeads.
18. Add pre-heated Elution Buffer (100 µL) and mix by vortexing for 10 seconds
19. Incubate the tube at 56 °C for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
20. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then transfer the cleared supernatant to a 1.5 ml RNase-free microcentrifuge tube.

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 ICGB buffer addition to the lysate, break up any precipitate as much as possible.
Eluted DNA does not perform well in downstream applications	➢ 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