ImaBeads® Genomic DNA Extraction Kit –Stool

For purification of genomic DNA from fresh or frozen stool samples.

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 %)*
- 2 ml microcentrifuge tubes
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 / 2 ml tubes)

* 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

ICGS 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:

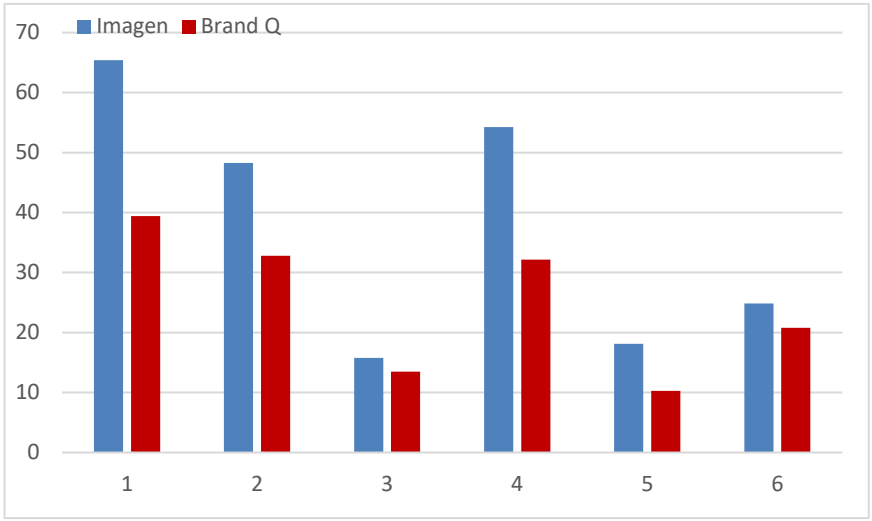
1. This kit should be stored at room temperature.
2. Proteinase K should be stored at 4 °C upon arrival.

Description

ImaBeads® Genomic DNA Extraction Kit – Stool is designed by patented technology for purification of total DNA (including genomic, mitochondrial and viral DNA) from fresh or frozen stool samples. The protocol uses buffer contains chaotropic salt to lyse cells and degrade protein. DNA will bind to magnetic beads. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer. Purified DNA of approximately 20-30 kb in length is suitable for PCR or other enzymatic reactions.

Using magnetic-particle technology to purify genomic DNA. The purified genomic DNA can be directly used for downstream applications, such as quantitative PCR, restriction enzyme digestion, southern blotting...etc.

ImaBeads® Genomic DNA Extraction Kit –Stool Test Data



(ng/uL)

DNA was purified from 6 stool samples using the ImaBeads® Genomic DNA Extraction Kit - Stool & Compeititive brand Q. Yields were measured by UV spectrophotometry. Yields were invariably highest with the ImaBeads® Genomic DNA Kit - Stool.

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.

Stool Protocol Procedure

1. Weigh up to 110 mg stool in a 2 ml microcentrifuge tube (not provided).
   This protocol is optimized for use with up to 110 mg stool but can also be used with smaller amounts. There is no need to reduce the amounts of buffers when using smaller amounts of stool. For samples >110 mg, please increase the buffer usage proportionally.
   If the sample is liquid, pipet 100 µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier.
2. Add 500 µl of ICGS Buffer and mix by vortexing for 10 seconds and incubate the tube at 90 °C for 10 minutes.
3. Centrifuge for 4 minutes a 13,000 rpm (10,000 x g) to pellet stool particles. Then carefully transfer 400ul supernatant to a new 1.5 ml microcentrifuge tube.
4. Add 20 µl of Proteinase K (10 mg/ml) to the tube and mix by vortexing and spin down and incubate the tube at 70 °C for 10 minutes.
   Optional RNA Removal Step:
   If RNA-free genomic DNA is required, perform this optional step.
   Following 70 °C incubation, cooling to room temperature, add 4 µl of RNase A (50 mg/ml) (not provided) to sample lysate and vortex to mix. Incubate at room temperature for 10 minutes.
5. At this time, preheat required Elution Buffer (100 µl per sample) in 56°C (For DNA Elution Step).
6. Vortex **ImaBeads – 01** to ensure they are in suspension prior to initial use and take 500ul **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 180 µl of ICGL Buffer to the sample lysate, vortex immediately for 10 seconds and incubate the tube at 56 °C for 10 minutes.
9. Add 380 µl of ICGB Buffer to the sample lysate, vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting.
10. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube containing the ImaBeads (prepared for use in step 7.) and mix with beads by vortexing for 10 minutes.
11. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
12. Add 800 µl of IBW1 Buffer and mix by vortexing for 1 minute.
13. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
14. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
15. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
16. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
17. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
18. Keep the cap open and incubate the tube at 56 °C for 2 minutes to dry the ImaBeads.
19. Add pre-heated Elution Buffer (100 µL) and mix by vortexing for 10 seconds.
20. Incubate the tube at 56 °C for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
21. 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