ImaBeads® Genomic DNA Extraction Kit –Tissue

For purification of genomic DNA from a variety to animal tissues, blood spots, feed-soil Sample**,** cigarette butts, hair roots, chewing gum**,** buccal swabs, betel nut residue, stool sample, saliva, sputum specimens, bacteria and cultured yeast.

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

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.

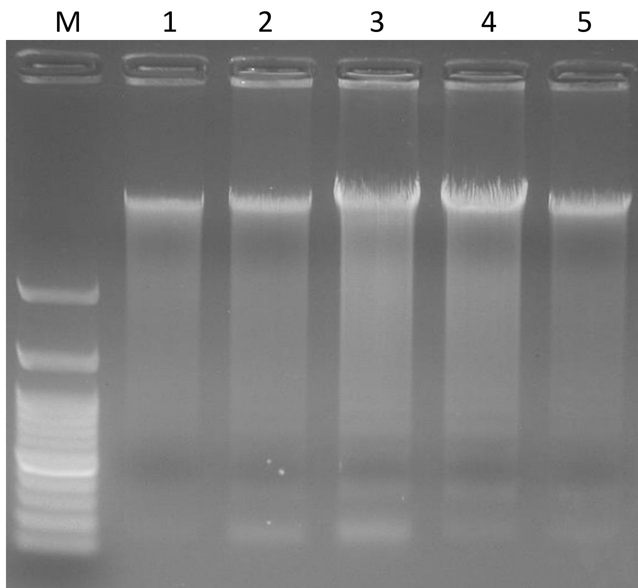
Description

ImaBeads® Genomic DNA Extraction Kit – Tissue is designed by patented technology for purification of total DNA (including genomic, mitochondrial and viral DNA) from animal tissues, feed-soil Sample**,** cigarette butts, hair roots, chewing gum**,** buccal swabs, betel nut residue, stool sample, saliva, sputum specimens, and bacteria and cultured yeast. 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 –Tissue Test Data



Genomic DNA from a variety of tissue samples was extracted using the ImaBeads® Genomic DNA Extraction Kit –Tissue. The purified genomic DNA was analyzed by electrophoresis on a 1.2 % agarose gel.
M = 1 Kb DNA Ladder
1 = Mouse heart 10 mg
2 = Mouse spleen 10 mg
3 = Mouse liver 10 mg
4 = Mouse Kidney 10 mg
5 = Rat tail 0.5 cm

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.

Tissue Protocol Procedure

1. Cut the solid tissue to small pieces (up to 25 mg) (or 0.5 cm of mouse tail) and transfer to a microcentrifuge tube (not provided). If tissue has a higher number of cells (e.g. spleen or liver), reduce starting material to 10mg.
2. Add 400 µl of ICGT Buffer and 20 µl of Proteinase K (10 mg/ml) to the tube and mix by vortexing.
3. Incubate at 56 °C overnight or until the sample has been completely lysed.
   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.
4. If insoluble material remains following incubation, centrifuge for 2 minutes a 13,000 rpm (10,000 x g) then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
5. 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.
7. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
8. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
9. 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.
10. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube (prepared for use in step 8.) 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. 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.

Amniotic Fluid Protocol Procedure

1. Transfer up to 15 ml of amniotic fluid to a 15 ml centrifuge tube. Centrifuge for 3 minutes at 14-16,000 x g then discard the supernatant.
2. Add 400 µl of ICGT Buffer and 20 µl of Proteinase K (10 mg/ml) to the tube and mix by vortexing.
3. Incubate at 56 °C overnight or until the sample has been completely lysed.
   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.
4. If insoluble material remains following incubation, centrifuge for 2 minutes a 13,000 rpm (10,000 x g) then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
5. 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.
7. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
8. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
9. 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.
10. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube (prepared for use in step 8.) 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. 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.

Hair roots Protocol Procedure

1. Cut the hair roots into 0.5 - 1 cm pieces.
2. Transfer the pieces to a 1.5 ml microcentrifuge tube. Add 400 µl of ICGT buffer and 20 µl of Proteinase K, close the lid, and mix for 10 seconds. Incubate at 56 °C for 1 hour or until the sample has been completely lysed.
   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.
3. Briefly centrifuge the sample tube to remove drops from the inside of the lid.
4. If insoluble material remains following incubation, centrifuge for 2 minutes a 13,000 rpm (10,000 x g) then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
5. Pipette 200 µl of clear sample solution to a new 1.5 ml RNase-free microcentrifuge tube.
6. Preheat required Elution Buffer (100 µl per sample) in 56°C (For DNA Elution Step).
7. Vortex **ImaBeads – 01** to ensure they are in suspension prior to initial use.
8. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
9. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
10. 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.
11. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube (prepared for use in step 9.) and mix with beads by vortexing for 10 minutes.
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 IBW1 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. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
18. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
19. Incubate the tube at 56 °C for 2 minutes to dry the ImaBeads.
20. Add pre-heated Elution Buffer (100 µL) and mix by vortexing for 10 seconds
21. Incubate the tube at 56 °C for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
22. 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.

Insect Protocol Procedure

1. Transfer up to 50 mg of insect tissue to a mortar. Add liquid nitrogen to the mortar and grind the tissue thoroughly using a pestle. Refill the mortar occasionally with liquid nitrogen to keep the sample frozen.
2. Transfer the tissue powder to a 1.5 ml microcentrifuge tube.
3. Add 400 µl of ICGT Buffer and 20 µl of Proteinase K then vortex thoroughly.
4. Incubate at 56 °C for 1-3 hours or until the sample lysate becomes clear.
   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.
5. Briefly centrifuge the sample tube to remove drops from the inside of the lid.
6. If insoluble material remains following incubation, centrifuge for 2 minutes a 13,000 rpm (10,000 x g) then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
7. Pipette 200 µl of clear sample solution to a new 1.5 ml RNase-free microcentrifuge tube.
8. Preheat required Elution Buffer (100 µl per sample) in 56°C (For DNA Elution Step).
9. Vortex **ImaBeads – 01** to ensure they are in suspension prior to initial use.
10. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
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 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.
13. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube (prepared for use in step 11.) and mix with beads by vortexing for 10 minutes.
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 IBW1 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. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
18. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
19. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
20. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
21. Incubate the tube at 56 °C for 2 minutes to dry the ImaBeads.
22. Add pre-heated Elution Buffer (100 µL) and mix by vortexing for 10 seconds
23. Incubate the tube at 56 °C for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
24. 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.

Bacterial Protocol Procedure

1. Harvest bacteria according to step I. (for Gram Negative) or II (for Gram Positive).
    I. Gram Negative
        A. Transfer bacterial culture (<10^9) to a 1.5 ml microcentrifuge tube (not provided).
        B. Centrifuge for 1 minute at 13,000 rpm (10,000 x g) and discard the supernatant.
        C. Add 400µl of ICGT Buffer and 20µl Proteinase K to the tube, and vortex or pipette to resuspend the cell pellet.
        D. Incubate at room temperature for 5 minutes.
    II. Gram Positive
        A. Prepare Lysozyme Buffer fresh immediately prior to use: 20mg/ml lysozyme; 20mM Tris-HCl; 2mM EDTA; 1% Triton X-100; pH 8.0
        B. Transfer bacterial culture (<10^9) to a 1.5 ml microcentrifuge tube (not provided).
        C. Centrifuge for 1 minute at 13,000 rpm (10,000 x g) and discard the supernatant.
        D. Add 400µl of Lysozyme Buffer and 20µl Proteinase K to the tube, and vortex or pipette to resuspend the cell pellet.
        E. Incubate at room temperature for 10 minutes. During incubation, invert tube every 2-3 minutes.
2. If insoluble material remains following incubation, centrifuge for 2 minutes a 13,000 rpm (10,000 x g) then carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube.
3. Preheat required Elution Buffer (100 µl per sample) in 56°C (For DNA Elution Step).
4. Vortex **ImaBeads – 01** to ensure they are in suspension prior to initial use.
5. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
6. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
7. 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.
8. Apply sample mixture to the 1.5 ml RNase-free microcentrifuge tube (prepared for use in step 6.) and mix with beads by vortexing for 10 minutes.
9. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
10. Add 800 µl of IBW1 Buffer and mix by vortexing for 1 minute.
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 IW2 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. Incubate the tube at 56 °C for 2 minutes to dry the ImaBeads.
17. Add pre-heated Elution Buffer (100 µL) and mix by vortexing for 10 seconds
18. Incubate the tube at 56 °C for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
19. 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 prior.
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