ImaBeads® Genomic DNA Extraction Kit –Blood & Cell

For purification of genomic DNA from whole blood, buffy coat, lymphocytes, body fluids, cultured cells

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

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 – Blood & Cell is designed by patented technology for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood, plasma, serum, buffy coat, up to $5x10^6$ cultured cells. 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 –Blood & Cell Test Data

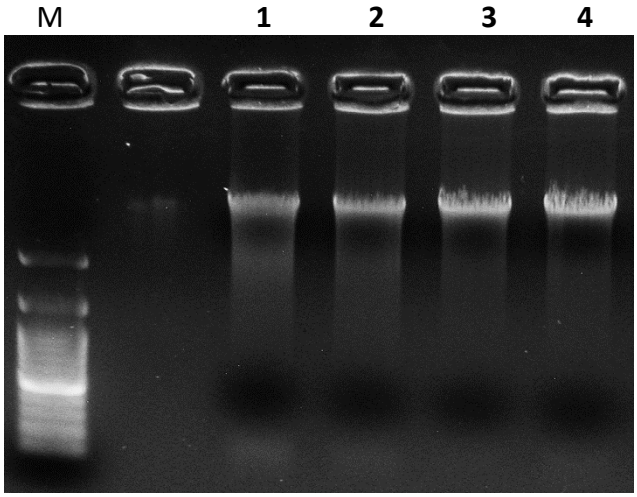

Fig 1. Whole Blood Genomic DNA extraction Comparison

Genomic DNA from 200 µl whole blood samples was extracted using the **ImaBeads® Genomic DNA Extraction Kit –Blood & Cell** and **Competitive Brand Q**. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 1 % agarose gel.

1-2 = Competitive Brand Q

3-4 = ImaBeads® Genomic DNA Extraction Kit –Blood & Cell

M = 1 Kb DNA Ladder

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.

Fresh Blood and body fluids Protocol Procedure

1. 200 µl Blood or Body Fluids add 20 µl Proteinase K (10 mg/ml) , mix by vortexing and 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.
   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. Add 180 µl of ICGL Buffer then shake vigorously for 10 seconds, Incubate at 56 °C for 5 minutes.
3. At this time, 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 380 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 5 minutes to dry the ImaBeads.
17. Add 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.

Blood-Buffy Coat Preparation by RBC Lysis Protocol Procedure

1. Take 200 µl whole blood into 2 ml microcentrifuge tube.
2. Add 600 µl RBC Lysis Buffer and mix together by inverting the tube.
3. Shake the mixture at 100 rpm for 5 minutes.
4. Centrifuge the mixture at 2,500rpm (500 x g) for 5 minutes.
5. Repeat step 2 ~ step 5 to wash the sample again.
6. Add 200 µl of PBS Buffer to resuspend the pellet
7. Add 20 µl of proteinase K, mix by vortexing and incubate at 56 °C for 5 minutes.
   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.
8. Add 180 µl ICGL Buffer, mix by vortexing and incubate at 56 °C for 5 minutes.
9. At this time, preheat required Elution Buffer (100 µl per sample) in 56°C (For DNA Elution Step).
10. Vortex **ImaBeads – 01** to ensure they are in suspension prior to initial use.
11. Take 500 µl of **ImaBeads – 01** to a 1.5 ml RNase-free microcentrifuge tube.
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 380 µl of ICGB Buffer to the sample lysate and vortex immediately for 10 seconds to mix sample. If precipitate appears, break up by pipetting.
14. 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.
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 IBW1 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. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
19. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
20. Add 800 µl of IW2 Buffer and mix by vortexing for 1 minute.
21. Place the tube in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
22. Incubate the tube at 56 °C for 5 minutes to dry the ImaBeads.
23. Add Elution Buffer (100 µL) and mix by vortexing for 10 seconds
24. Incubate the tube at 56 °C for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
25. 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.

Blood-Buffy Coat Preparation by Centrifugation Protocol Procedure

1. Take 2 - 5 ml of whole blood sample and centrifuge at 2,500rpm (500 x g) rpm for 10 minutes.
2. Use plastic dropper to take white buffy coat layer in the middle of whole blood sample and move the buffy coat into a new microcentrifuge tube.
3. Take 40-50 µl buffy coat sample into a 1.5 ml centrifuge tube and add PBS until 200 µl then add 20µl of proteinase K, mix by vortexing and 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.
   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. Add 180 µl ICGL Buffer and mix by vortexing, incubate at 56 °C for 5 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.
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 380 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 6.) 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 5 minutes to dry the ImaBeads.
19. Add 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.

Cultured Cell Protocol Procedure

1. Harvest cells according to step I. (for cells grown in suspension) or II & III. (for cells grown in a monolayer).
   I. Cells grown in suspension (do not use more than 5 x 10^6 cells with a normal set of chromosomes): Determine the number of cells. Centrifuge the appropriate number of cells for 5 minutes a 2,500rpm (500 x g) in a 1.5 ml microcentrifuge tube. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.
   II. Cells grown in a monolayer (do not use more than 5 x 10^6 cells with a normal set of chromosomes): Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper. To trypsinize cells: Determine the number of cells. Aspirate the medium and wash cells with PBS. Aspirate the PBS, and add 0.10–0.25 % trypsin. After cells have detached from the dish or flask, collect them in medium, and transfer the appropriate number of cells (do not use more than 5 x 10^6 cells with a normal set of chromosomes) to a 1.5 ml microcentrifuge tube. Centrifuge for 5 minutes at 2,500rpm (500 x g). Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.
   III. Using a cell scraper: Detach cells from the dish or flask. Transfer the appropriate number of cells (do not use more than 5 x 10^6 cells with a normal set of chromosomes) to a 1.5 ml microcentrifuge tube and centrifuge for 5 minutes at 300 x g. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Continue with step 2.
1. Add 200 µl PBS Buffer and 20 µl Proteinase K (10 mg/ml) to the tube and mix by vortexing.
2. Incubate at 56 °C for 5 minutes or until the sample has been completely lysed.
   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.
   Optional RNA Removal Step:
   If RNA-free genomic DNA is required, perform this optional step.
   Following 56 °C incubation (step 3.), 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. Add 180 µl of ICGL Buffer then shake vigorously for 10 seconds, Incubate at 56 °C for 5 minutes.
4. At this time, 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 380 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 8.) 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 5 minutes to dry the ImaBeads.

18. Add 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 ethanol addition, break up any precipitate as much as possible.
Eluted DNA does not perform well in downstream applications	➢ 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