ImaBeads® Total RNA Extraction Kit –Blood & Cell

For total RNA extraction from whole blood & 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
- β - Mercaptoethanol (β - ME)
- DNase I (optional)

*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 IRW2 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

RBC Lysis Buffer
ISRL Buffer
ISRB Buffer
IRW1 Buffer
IRW2 Buffer (Add Ethanol)
RNase-free Water
ImaBeads-10

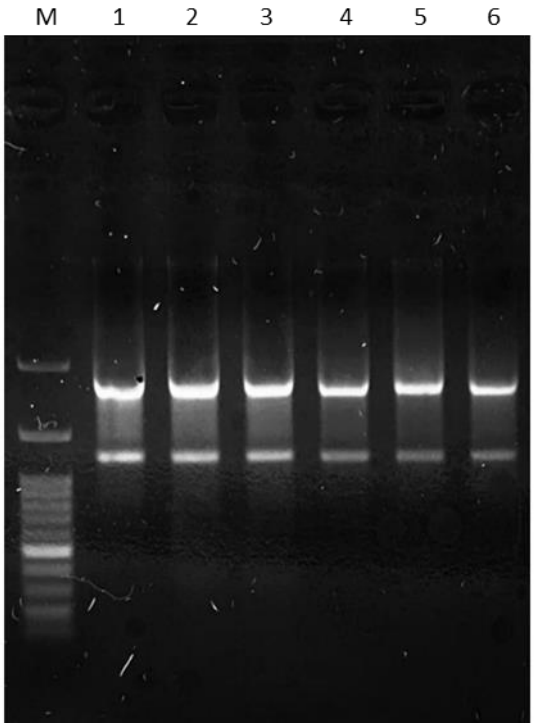
Storage and Stability:

This kit should be stored at room temperature.

Description

ImaBeads® Total RNA Extraction Kit –Blood & Cell is designed by patented technology for purification of total RNA from bacterial, cultured cells and fresh human whole blood. The method uses detergents and a chaotropic salt to lyse the cells and inactivate RNase. RNA in chaotropic salt solutions binds to the ImaBeads matrix . Following washing off of contaminants, the purified RNA is eluted by RNase-free water. ssRNA and dsRNA can be efficiently purified. Purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.

ImaBeads® Total RNA Extraction Kit –Blood & Cell Test Data



Triple repeat of total RNA extraction from cultured 5 x 10^5 & 1 x 10^5 HeLa Cell samples using the ImaBeads® Total RNA Extraction Kit – Whole Blood & Cell. 10 µl from 60 µl eluates of purified Total RNA was analyzed by electrophoresis on a 1% agarose gel.

M = 1 Kb DNA Ladder
1-3 = 5 x 10^5
4-6 = 1 x 10^5

Preparation before using

1. Total RNA Extraction Kit has been optimized for preferential RNA binding, however genomic DNA contamination is almost impossible to avoid during RNA extraction procedures. DNase I (RNase-Free) may be applied to the binding column according to the protocol instructions. It is necessary to use highly purified DNase. If RNase is present in trace amounts it will result in RNA degradation. It is recommended to apply DNase for sensitive downstream applications, however for many downstream applications it may not be necessary to apply as genomic DNA contamination may be negligible or inconsequential to the application.
2. Wear a lab coat and disposable gloves to prevent RNase contamination.
3. Before use, add 10 µl of β - ME to 1 mL of ISRL Buffer. ISRL Buffer Containing β - ME can be stored at room temperature for up to 1 month.

Whole Blood Protocol Procedure

1. Add 400 µl volume of human whole blood with 2000 µl of RBC lysis Buffer in an appropriately sized tube (not provided) and mix completely by inversion. Do not vortex.
   Note: For optimal results, the volume of the mixture (blood + RBC lysis Buffer) should not exceed 4/5 of the volume of the tube to allow efficient mixing.
2. Incubate the tube for 10 minutes on ice and invert 3 times during incubation.
3. Centrifuge for 5 minutes at 2,500 rpm (500 x g) at 4°C and completely discard the supernatant.
4. Add 400 µl of RBC lysis Buffer to the cell pellet. Resuspend cells by vortex briefly.
5. Incubate the tube for 3 minutes on ice.
6. Centrifuge for 5 minutes at 2,500 rpm (500 x g) at 4 °C and completely discard the supernatant.
7. Add 400 ul of ISRL Buffer (contain β-ME) to the white pellet and mix by vortexing.
8. Incubate at room temperature for 5 minutes.
9. Vortex **ImaBeads – 10** to ensure they are in suspension prior to initial use.
10. Take 500 µl of **ImaBeads –10** to a 1.5 ml RNase-free microcentrifuge tube.
11. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then discard the cleared supernatant and remove the tube.
12. Add 400 ul of ISRB Buffer to the sample lysate and mix by pipetting immediately for 10 seconds.
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.
    Optional: If performing optional DNase digestion
15. Add 800 µl of IBW1 Buffer and mix by vortexing for 1 minute.
16. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then discard the cleared supernatant and remove the tube.
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 room temperature for 5 minutes to dry the ImaBeads.
22. Add RNase-free Water (100 µL) and mix by vortexing for 10 seconds.
23. Incubate the tube at room temperature for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
24. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then transfer the cleared supernatant to a new 1.5 ml RNase-free microcentrifuge tube.

Cultured Animal Cells Protocol Procedure

1. Transfer maxim 5x 10^6 of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation for 5 minute at 2,500 rpm (500 x g).
2. Discard the supernatant.
3. Add 400 µl of ISRL Buffer to the sample tube and mix by vortexing.
4. Incubate at room temperature for 5 min.
5. Vortex **ImaBeads – 10** to ensure they are in suspension prior to initial use.
6. Take 500 µl of **ImaBeads –10** to a 1.5 ml RNase-free microcentrifuge tube.
7. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then discard the cleared supernatant and remove the tube.
8. Add 400 ul of ISRB Buffer to the sample lysate and mix by pipetting immediately for 10 seconds.
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.
    Optional: If performing optional DNase digestion
11. Add 800 µl of IBW1 Buffer and mix by vortexing for 1 minute.
12. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then discard the cleared supernatant and remove the tube.
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 room temperature for 5 minutes to dry the ImaBeads.
18. Add RNase-free Water (100 µL) and mix by vortexing for 10 seconds.
19. Incubate the tube at room temperature for 10 minutes and mix by vortexing for 10 seconds per 3 minutes.
20. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then transfer the cleared supernatant to a new 1.5 ml RNase-free microcentrifuge tube.

Optional Step:

DNA residue degradation Procedure

1. Add 200 µl of Buffer IRW1 Buffer to the 1.5ml tube. and mix by vortexing for 1 minute to wash the ImaBeads.
2. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then discard the cleared supernatant and remove the tube.
3. Add 10 µl of DNase I stock solution (3 Kunitz units / µl) to 70 µl of Buffer DNase I Reaction Buffer. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.
   Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.
4. Add the DNase I mixture (80 µl) directly to sample tube, and incubate at 20 – 30 °C for 15 minutes.
5. Add 200 µl of Buffer IRW1 Buffer. Close the lid gently, and mix by vortexing for 1 minute to wash the ImaBeads.
6. Place the tube on a magnetic separator for 1 minute or until ImaBeads have pelleted then discard the cleared supernatant and remove the tube.
7. Continue with the IRW1 Buffer wash step.

Troubleshooting

Problem	Possible Reasons/Solution
DNA contamination	➢ Perform in Column DNase I Digestion to eliminate DNA contamination.
Eluted RNA does not perform well in downstream applications	➢ Residual Ethanol Contamination: Following the wash step, Incubate the tube at room temperature for 5 minutes to dry the ImaBeads.
Low RNA Yield	➢ Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. ➢ Insufficient disruption and homogenization/too much starting material, try to adjust it. ➢ RNA still bound to the IR Column membrane, elute twice to increase the yield. ➢ Ethanol carryover; following the wash step, Incubate the tube at room temperature for 5 minutes to dry the ImaBeads. Ensure RNase-free Water is added into the CENTER of the Imabeads matrix.
RNA Degradation	➢ Harvested sample immediately stabilized/inappropriate handling of starting material. ➢ Avoid RNase contamination by always wear gloves & mask and treat all the equipment with RNaseOUT.