ImaBeads® Total RNA Extraction Kit –Tissue

For purification of total RNA from a variety to Tissue

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
- ß - Mercaptoethanol (ß - ME)

* 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

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

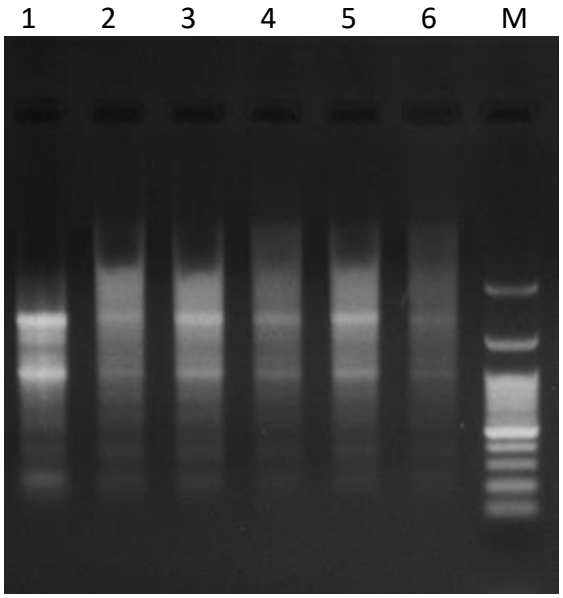
Storage and Stability:

This kit should be stored at room temperature.

Description

ImaBeads® Total RNA Extraction Kit – Tissue is designed by patented technology for purification of total RNA from a variety of animal tissue. The protocol uses buffer contains chaotropic salt to lyse cells and inactivate RNase. RNA will bind to magnetic beads. After washing off the 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 –Tissue Test Data



Total RNA from 10 mg Rat tissue samples was extracted using ImaBeads® Total RNA Extraction Kit - Tissue. 10 µl from 60 µl eluates of purified Total RNA was analyzed by electrophoresis on a 1.5 % agarose gel.

1 = Rat liver 10mg
2 = Rat thymus 10mg
3 = Rat spleen 0.5 cm
4 = Rat muscle 10mg
5 = Rat Kidney 10mg
6 = Rat lung 10mg
M = 1 Kb DNA Ladder

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 ß-ME to 1 mL of IRL Buffer. IRL Buffer Containing ß-ME can be stored at room temperature for up to 1 month.

Tissue Protocol Procedure

1. Cut off 10mg fresh or forzon animal tissue 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 ISRL Buffer (ß-ME added) into the tube and use provided micropestle to sufficiently grind the tissue a few times.
3. Incubate at room temperature for 5 minutes.
4. If insoluble material remains following incubation, centrifuge for 2 minutes at 13,000 rpm (10,000 x g) and transfer all the clarified supernatant to a new microcentrifuge tube (not provided).
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 in a magnetic separator for 1 minute or until ImaBeads have pelleted then remove and discard the cleared supernatant.
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.
11. Add 800 µl of IRW1 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 IRW2 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 IRW2 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. 三階寫"室溫以vortexer震動混合10分鐘"
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 RNA Yield	➢ Ensure absolute ethanol was added to IRW2 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.
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.