

ImaSpin® Total RNA Extraction Kit –Tissue

For total RNA extraction from 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
- β - Mercaptoethanol (β - ME)
- DNase I (optional)
- 20-G needle (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

IRL Buffer

IRB Buffer

IRW1 Buffer

IRW2 Buffer (Add Ethanol)

RNase-free Water

IR Column

Micropestle

2 ml Collection Tubes

Storage and Stability:

This kit should be stored at room temperature.

Description

ImaSpin® Total RNA Extraction Kit –Tissue is designed by patented technology for purification of total RNA from a variety of animal tissue. The method uses detergents and a chaotropic salt to lyse the cells and inactivate RNase. RNA in chaotropic salt solutions binds to the glass fiber matrix of the IR columns. 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.

ImaSpin® Total RNA Extraction Kit –Tissue Test Data

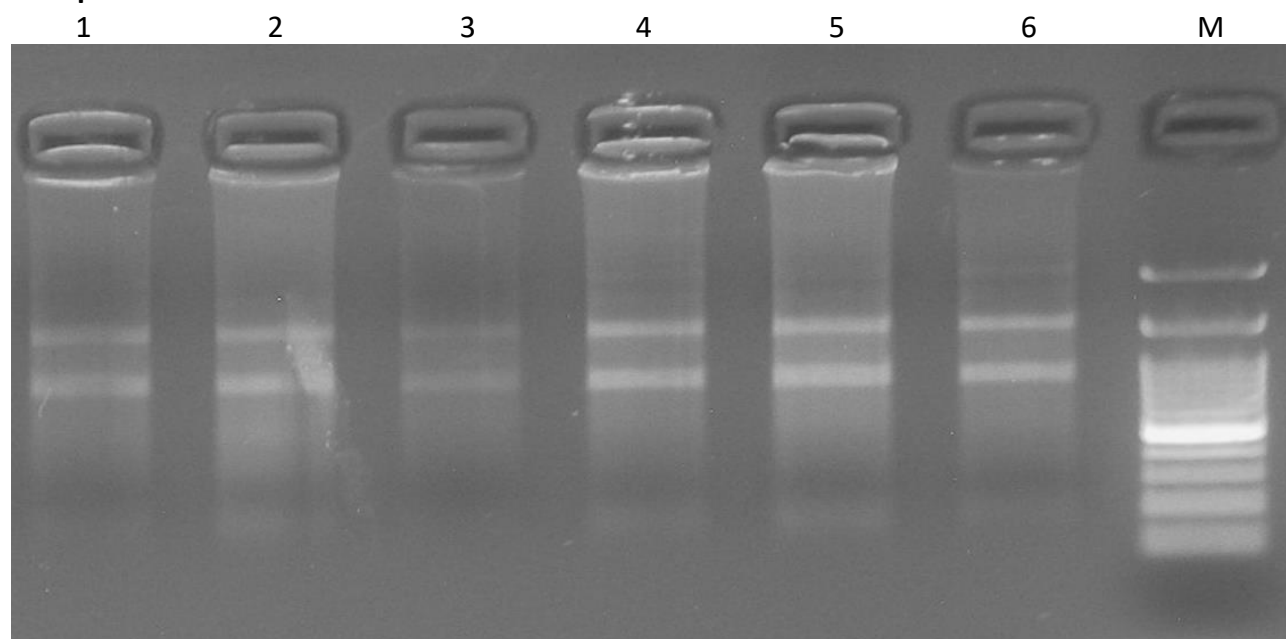


Fig 1. Total RNA extracted from Rat tissue

Total RNA from 10 mg of Rat tissue samples was extracted using the ImaSpin® 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 = Muscle
- 2 = Kidney
- 3 = Lung
- 4 = Thymus
- 5 = Liver
- 6 = Spleen
- 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 μ l of β -ME to 1 mL of IRL Buffer. IRL Buffer Containing β -ME can be stored at room temperature for up to 1 month.

Tissue Protocol Procedure

1. Cut off 10 mg of fresh or frozen animal tissue and transfer it into a RNase-free microcentrifuge tube (not provided).
2. Add 400 μ l of IRL Buffer (β -ME added) into the tube and use provided micropestle to sufficiently grind the tissue a few times.
3. Shear the tissue by passing lysate through a 20-G needle syringe 10 times. (optional)
4. Incubate at room temperature for 5 minutes.
5. 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).
6. Add 400 μ l of IRB Buffer to the sample lysate and mix by pipetting immediately for 10 seconds.
7. Place a IR Column in a 2 ml Collection Tube. Transfer all of the mixture to the IR Column.
8. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes.
9. Discard the flow-through then place the IR Column back in the 2 ml Collection Tube.
Optional: If performing optional DNase digestion
10. Add 600 μ l of IRW1 Buffer to the IR Column.
11. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IR Column back in the 2 ml Collection Tube.
12. Add 600 μ l of IRW2 Buffer (make sure absolute ethanol was added) to the IR Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute then discard the flow-through. Place the IR Column back in the 2 ml Collection Tube.
14. Add 600 μ l of IRW2 Buffer (make sure absolute ethanol was added) to the IR Column.
15. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute then discard the flow-through. Place the IR Column back in the 2 ml Collection Tube.
16. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
17. Transfer the dried IR Column to a clean 1.5 ml microcentrifuge tube.
18. Add 50 μ l of RNase-free Water into the CENTER of the column matrix.
19. Let stand for at least 2 minutes to allow Elution Buffer or DEPC water to be completely absorbed.
20. Centrifuge at 13,000 rpm (10,000 x g) for 2 minute to elute the purified RNA.

Optional Step:

DNA residue degradation Procedure

1. Add 200 μ l of Buffer IRW1 Buffer to the IR column. Close the lid gently, and centrifuge for 15 seconds at 13,000 rpm (10,000 x g) to wash the spin column membrane. Discard the flow-through.
2. Add 10 μ l of DNase I stock solution (3 Kunitz units / μ l) to 70 μ l of 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.
3. Add the DNase I mixture (80 μ l) directly to sample tube, and incubate at 20 – 30 °C for 15 minutes.
4. Add 200 μ l of Buffer IRW1 Buffer. Close the lid gently, and centrifuge for 15 seconds at 13,000 rpm (10,000 x g) to wash the spin column membrane. Discard the flow-through.
5. 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, dry the IR Column with additional centrifugation at 13,000 rpm (10,000 x g) for 5 minutes.
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, dry the IR Column with additional centrifugation at 13,000 rpm (10,000 x g) for 5 minutes. ➤ Ensure RNase-free Water is added into the CENTER of the column 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.