Ima96 ® 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
- ß - Mercaptoethanol (ß - 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 PRW2 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
PRL Buffer
PRB Buffer
PRW1 Buffer
PRW2 Buffer (Add Ethanol)
RNase-free Water
Ima96 Plates
Tape Sheets
96 Deep Well Plates
0.35 ml Collection Plates

Storage and Stability:

This kit should be stored at room temperature.

Description

Ima96 ® 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 glass fiber matrix of the Ima96 plates. 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.

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

Cultured Animal Cells Protocol Procedure

1. Transfer maxim 5x 10^5 of cells to a microcentrifuge tube (not provided) and harvest the cells with centrifugation for 5 minutes at 2,500 rpm (500 x g) at 4°C and discard the supernatant
2. Prepare the vacuum manifold. Place 96 Deep well plate in the vacuum manifold base and position the Pall NAB plate on top of the manifold collar.
3. Add 200 ul of PRL buffer (contain β-ME) to the white pellet and mix by vortexing.
4. Add 200 ul of PRB buffer to the sample lysate and mix by pipetting immediately for 10 seconds.
5. Transfer the lysates to corresponding wells of the Pall NAB plate.
6. Apply vacuum for one minute until all lysate has passed through the Pall NAB plate.
   Optional: If performing optional DNase digestion
7. Add PRW1 Buffer (800 µL/well) and apply vacuum for one minute until all lysate has passed through the Pall NAB plate.
8. Discard the filtrate from 96 Deep well plate. The 96 Deep well plate can be reused.
9. Add PRW2 Buffer (800 µL/well) and apply vacuum for 20 seconds until all lysate has passed through the Pall NAB plate.
10. Add PRW2 Buffer (800 µL/well) and apply vacuum for 20 seconds until all lysate has passed through the Pall NAB plate.
11. Discard the filtrate from 96 Deep well plate. The 96 Deep well plate can be reused.
12. Lift the Pall NAB plate from the manifold collar, and strike the bottom side of the Pall NAB plate on a stack of paper towels (~4 cm high). Repeat several times until no further liquid is released onto the paper towels.
13. Place the Pall NAB plate back in the manifold collar.
14. Apply vacuum for 10 minutes.
15. Replace the 96 Deep well plate with an 0.35 ml Collection Plates assembled on top of the Elution Microtube Adapter.
16. To elute, pipet 60 µl of RNase-free water directly onto the membrane in each well. Let stand for 2 min.
17. Apply vacuum for 1 minute until transfer is complete.

Optional Step:

DNA residue degradation Procedure

1. Add 200 µl of Buffer PRW1 Buffer to the Pall NAB plate.
2. Apply vacuum for one minute until all lysate has passed through the Pall NAB plate.
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 PRW1 Buffer.
6. Apply vacuum for one minute until all lysate has passed through the Pall NAB plate.
7. Discard the filtrate from 96 Deep well plate. The 96 Deep well plate can be reused.
8. Continue with the PRW1 Buffer wash step.

Troubleshooting

Problem	Possible Reasons/Solution
DNA contamination	➢ Perform in 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 Ima96 plate with additional vacuum for 5 minutes until transfer is complete.
Low RNA Yield	➢ Ensure absolute ethanol was added to PRW2 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 Ima96 membrane, elute twice to increase the yield. ➢ Ethanol carryover; following the wash step, dry the Ima96 with additional vacuum for 5 minutes until transfer is complete. ➢ Ensure RNase-free Water is added into the CENTER of the Ima96 plate 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.