MQ Tissue RNA Extraction Kit

Cat No. TRE-M-001-50
Size: 50 preps

MOLEQULE-ON®

Components	TRE-M-001-50 (50 Preps)
Buffer RLT	25 ml
Buffer RW	30 ml
Universal RPE Solution*	12 ml
RNase Free Water	5 ml
MQ Spin Column with 2.0ml Collection Tubes	50

Preparation

*Universal RPE Solution is supplied as a concentrate. Before use, add 48ml of 96-100% ethanol to 12 ml concentrated universal RPE solution and mix well.

Description

MQ Tissue RNA Extraction Kit allows efficient extraction of total RNA from various samples. Total RNA is easily purified from animal or human cells and tissues using a simple spin format. This kit simplifies total RNA isolation by combining the stringency of guanidine-isothiocyanate lysis with the speed and purity of silica-based purification. Samples are first lysed and then homogenized. Ethanol is added to the lysate to provide optimal binding conditions. The lysate is then loaded onto the MQ column with a silica membrane. While the RNA binds to the silica membrane, all proteins and other components are removed in the flow-through. Remaining contaminants and salts are efficiently washed away. The purified RNA eluted in RNase-free water has $OD_{260/280}$ ratios of 1.9-2.1 (measured in 10 mM Tris HCl, pH 7.5) and is ideal for use in most downstream applications including Northern blotting, RT-PCR, Quantitative PCR, Poly (A) RNA selection and Array analysis.

Storage

Store MQ Tissue RNA Extraction Kit at 4°C.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- Pipettes and pipette tips
- Vortexer
- Ethanol (96-100%)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Water bath for heating at 65°C

Protocol

1. Sample preparation

A. Adherent cells:

Do not use more than 1 x 10^7 cells. Cells can be either lysed directly in the cell culture petri-dish or trypsinized and collected as a cell pellet prior to lysis. For direct lysis of cells grown in a monolayer, add 0.45 ml Buffer RLT to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 2.

To trypsinize and collect cells:

Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. Aer the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free polypropylene centrifuge tube (not supplied), and centrifuge at 300 x g for 5 min. Completely aspirate the supernatant. Add 0.45 ml Buffer RLT per 5 cm^2 cultured cell. Mix gently by pipetting up and down several times.

B. Suspension cells:

Collect cells by centrifuge, discard the supernatant. Loosen the cell pellet thoroughly by flicking the tube. Add 0.45 ml Buffer RLT for 1-5 × 106 cells, mix gently by pipetting. The amount of cells should not exceed 1 × 10^6 for fibroblasts or carcinoma cells.

C. Fresh Tissue:

Cut the tissue into pieces and grind to fine powder in liquid nitrogen. Add 0.45 ml Buffer RLT for 25-50 mg tissue, homogenate for 30 sec using a rotor-stator homogenizer. Alternatively, one can pass the lysate at least 5 times through a blunt 20-Gauge needle (0.9mm diameter) fitted to an RNase-free syringe. Proceed to step 2.

2. Add 1/2 volume of ethanol, mix by inverting the tube. Do not centrifuge.

3. Transfer the solution including any precipitate that may have formed, to the spin column placed in a 2ml collection tube. Centrifuge at 12,000 × g for 30 sec at room temperature, discard the flow-through.

4. Add 0.5 ml of RW Solution to the column, centrifuge at 12,000 × g for 30 sec at room temperature, discard the flow-through.

Note: If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same MQ spin column. Discard the flow-through after each centrifugation.

5. Add 0.5 ml of Universal RPE Solution to the column, centrifuge at 12,000 × g for 30 sec at room temperature, discard the flow-through.

Note: Universal RPE Solution is supplied as a concentrate. Before use, add 48ml of 96-100% ethanol to 12 ml concentrated universal RPE solution and mix well.

6. Centrifuge the column at 12,000 × g for 30 sec at room temperature.

Note: This step is very important to remove the residual ethanol thoroughly.

7. Place the column to a new 1.5 ml centrifuge tube; add 50 µl RNase-free Water. Keep at room temperature for 2 minutes. Centrifuge at 12,000 × g for 30 sec at room temperature, save the eluted RNA solution at -80°C.

Note: Care must be taken when working with RNA. It is important to maintain an RNase-free environment, starting with RNA sample preparation and continue through.