MQ Tissue RNA Extraction Kit (with gDNA Eliminator Column)

Cat No. TRE-M-001-50g
Size: 50 Preparations

MOLEQULE-ON®

Components	Quantity
Buffer Lysis DR	20 ml
GT Solution (concentrate)*	18 ml
NT Solution (concentrate)*	6 ml
RNase Free Water	5 ml
gDNA Eliminator Column with 2ml Collection Tubes	50 each
MQ Columns and 2.0 Collection Tubes	50 each

Preparations

- Salt precipitation in Buffer Lysis DR and GT Solution (concentrate) may occur at low temperature. Re-dissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.
- GT Solution and NT Solution are supplied as concentrates.
- Before the first use, add ethanol to GT and NT solutions in below mentioned volume.
- ***In 18ml GT Solution, add 12ml of 96-100% Ethanol to make a total volume of 30ml.**
- ***In 6ml NT Solution, add 24ml of 96-100% Ethanol to make a total volume of 30ml.**
- 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 purification and analysis. Use RNase free tubes, tips, gels. Wear gloves at all the time.

Description

MQ Tissue RNA Extraction Kit (with gDNA Eliminator Column) is designed to purify RNA from small amounts of animal cells or tissues. Samples are lysed and homogenized by Buffer Lysis DR. Genomic DNA contamination is effectively removed using a specially gDNA Eliminator Column. RNA is absorbed on MQ RNA Column. Finally, the RNA is eluted from MQ RNA Column. The purified RNA is ready to use and is ideally suited for downstream applications that are sensitive to low amounts of DNA contamination, such as quantitative, real-me RT-PCR. The procedure is simple and fast, requires no need for phenol/chloroform extraction. The whole procedure takes less than 30 minutes.

Features

• Provides efficient gDNA Eliminator Column to remove DNA during RNA purification.
• Completely removal of DNA contaminant.
• The entire procedure can be completed in 30 minutes.
• High yield and reproducibility.
• No phenol/chloroform extraction or ethanol precipitation required.
• High-purity RNA is suited for downstream applications that are sensitive to low amounts of DNA contamination.

Storage

The Kit should be store at room temperature (15-25°C).

Materials and Equipment Required but Not Supplied

- Microcentrifuge capable of at least 12,000 × g
- RNase-free pipets and pipette tips
- RNase-free microcentrifuge tubes (1.5 ml or 2 ml)
- Vortexer
- Ethanol (96-100%)

Procedure

1. Sample preparation:

A. Cell Cultures:

1a. Cells grown in suspension: Spin appropriate number of cells (max. 1 x 10^7) at 300 x g for 5 minutes at room temperature. Remove supernatant carefully, proceed to step 2.
1b. Cells grown in monolayer: Aspirate the medium and add 350 µl Buffer Lysis DR to the cell-culture dish. Collect the lysate with a rubber policeman. Pipette the lysate into a microcentrifuge tube. Vortex or pipette to mix, and ensure that no cell clumps are visible before proceeding to step 2.
• If sample can not be used immediately for genomic DNA extraction, it is recommended to store at -80°C for long-term.
• Avoid repeated freezing and thawing of stored samples, as this leads to RNA degradation.

B. Animal Tissue:

Grind 15~30 mg animal tissue to fine powder in liquid nitrogen. Transfer the powder to 1.5 ml RNase-free centrifuge tube.

C. Plant:

Grind 25~50 mg plant tissue to fine powder in liquid nitrogen. Transfer the powder to 1.5 ml RNase-free centrifuge tube.

2. Add 350 µl Buffer Lysis DR immediately to the 1.5 ml RNase-free centrifuge tube above, mix by vortex. Incubate the mixture for 5 minutes at room temperature.

3. Place the gDNA Eliminator Column in a 2 ml collection tube. Transfer the lysate to the column, keep at room temperature for 1 minute, centrifuge at 9,000 x g for 1 minute at room temperature.

4. Transfer the flow-through to a new RNase-free tube for RNA purification.

5. Add 250 µl ethanol to the flow-through from step 4, mix thoroughly.

6. Place the MQ RNA Column in the collection tube and transfer the mixture from step 5 to the MQ RNA Column, centrifuge at 9,000 x g for 1 minute at room temperature.

7. Place the MQ RNA Column in the collection tube, add 500 µl GT Solution, keep at room temperature for 1 minute, centrifuge at 9,000 x g for 1 minute at room temperature. Discard the flow-through.
• Check the label to ensure GT Solution was diluted with ethanol.

8. Add 500 µl NT Solution to the column, keep at room temperature for 1 minute. Followed by centrifuge at 9,000 x g for 1 minute at room temperature. Discard the flow-through.
• Check the label to ensure NT Solution was diluted with ethanol.

9. Place the column in the collection tube, and centrifuge at 9,000 x g for 2 minutes at room temperature.

10. Transfer the column into a new RNase-free centrifuge tube, and open it until the ethanol has completely evaporated (about 3-5 minutes).
• It is important to dry the membrane of the MQ RNA Column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

11. Add 30-50 µl RNase-free water, keep at room temperature for 2 minutes, and centrifuge at 9,000 x g for 2 minutes.
• The solution in the centrifuge tube is the RNA sample, it can be used immediately for downstream molecular operation or stored at -70°C. 12. Purified RNA is ready to use, keep at -80°C for long term storage.

Troubleshooting Guide

1. Clumping in binding steps

A. Make sure samples are homogenized completely before binding the spin column.
B. Reduce the amount of starting material.

2. RNA degradation

A. Use fresh sample. For frozen samples, ensure that they were frozen immediately in liquid nitrogen and properly stored at -70°C.
B. We recommend treating the tissue samples with liquid nitrogen and proceeding to sample lysis immediately.
C. Create an RNase-free working environment.
D. Wear gloves during all steps of the procedure. Change gloves frequently.
E. Use of RNase-free microcentrifuge tubes is recommended.

3. Low yield

A. Homogenize tissue completely.
B. Use advisable amount of starting material.
C. Check the label to ensure GT Solution and NT Solution were diluted with ethanol.
D. Elution step has to be strictly followed.

4. Inhibition of downstream enzymatic reactions

A. Residual ethanol from GT Solution and NT Solution can inhibit downstream enzymatic reactions. Centrifuge the column at 12,000 × g for 2 minutes to remove the residual ethanol thoroughly.
B. Residual salt can inhibit downstream enzymatic reactions. Ensure that GT Solution, NT Solution have been used at room temperature (15–25°C).

5. DNA residual

A. Reduce the amount of starting material.
B. Complete removal of cell-culture medium or stabilization reagent.