MQ RNA Isolation Kit

Cat No. RIK-M-001-50
Size: 50 Preparations

MOLEQULE-ON®

Components	Quantity
Lysis buffer (RL)	25 ml
Wash buffer 1 (RW1)	35 ml
Wash buffer 2 (RW2)	10 ml
Elution buffer (RE) RNase free water	15 ml
MQ RNA binding column (green, with collection tubes)	50
1.5 ml collection tubes	50
2 ml collection tubes	100

Storage

Store the MQ RNA Isolation kit at room temperature (15-25°C).

Materials and Equipment Required but Not Supplied

- Reducing agent: DTT or TCEP or 2-Mercaptoethanol (2-ME)
- Freshly prepared 70 % ethanol
- 96-100 % ethanol
- Sterile Gloves
- Sterile, RNase-free pipet tips
- 1.5 ml reaction tubes
- Equipment: pipette, centrifuge, heat block, vortex mixer, homogenizer

Description

The MQ RNA Isolation Kit purifies total RNA samples from mammalian tissues and cultured cells. The isolated RNA can be used for a variety of downstream applications e.g. RT-PCR, qPCR, cDNA synthesis, northern blot, next generation sequencing and much more. In order to prevent RNA degradation the sample is treated right at the beginning with an RNases inhibitory lysis buffer RL. This step ensures purification of intact RNA. Addition of ethanol provides appropriate RNA binding conditions to the silica membrane of the MQ binding column. In following steps contaminations are efficiently washed away with the supplied buffers RW1 and RW2 from the column. High-quality RNA is subsequently eluted in 50 µl RE buffer. The purified RNA is ready for downstream applications or can be stored at -70°C in a freezer.

Preparation of Working Solutions

1. Lysis buffer RL

Add one of the below listed reducing agents to buffer RL only at the following ratio according to the number of samples.

i. Final concentration of DTT: 40 mM
ii. Final concentration of TCEP-HCl: 20 mM
iii. Final concentration of 2-Mercaptoethanol: 1% (v/v)*

Reductant	Volume of Reductant	Volume of Buffer RL	Final Conc. of Reductant
2M DTT	20 µl	1 ml	40 mM
1M TCEP	20 µl	1 ml	20 mM
2-ME	10 µl	1 ml	1%*

- 2-ME is generally sold with a concentration of 14.3 M, the final concentration of 1% is 143 mM in terms of molar concentration.

Preparation of Working Solutions

2. Second wash buffer RW2

Add 40 ml of 96-100% ethanol to 10 ml RW2 and mix.

Sample Preparation

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore, it is important that samples are flash frozen in liquid N2 immediately and stored at -70°C or processed as soon as possible with the MQ RNA Isolation Kit. Cultured animal cells are collected by centrifugation and directly lysed by adding lysis buffer RL1 according to the protocol (step 2). Make sure that the cell culture medium is removed completely before adding Lysis buffer RL1. Animal tissues are often solid and must therefore be broken up mechanically as well as lysed. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption.
The most commonly used technique for disruption of animal tissues is grinding with a pestle and mortar or using the MOLEQULE-ON Tissue Homogenizer. Grind the sample to a fine powder in the presence of liquid N2. Take care that the sample does not thaw during or after grinding or weighing and add the frozen powder to an appropriate aliquot of Lysis buffer RL1 containing reducing agent. Depending on the amount of starting material, the viscosity of the lysed sample has to be reduced further for optimal results by passing the lysed sample > 10 times through a 0.9 mm syringe needle or by using the MQ Filter columns included in the Advanced kit. Make sure not to use higher amount of starting material since that can decrease yield and purity of the eluated RNA.

Procedure

Before starting the purification, please ensure that the following preparations have been made according to the above mentioned procedures:
• Reductant is added to lysis buffer RL
• Addition of ethanol to buffer RW2

1. Harvest samples in a reaction tube (not provided in the kit). Proceed the next step as quickly as possible.

	Standard	Large Input
Cells	~5 x 10^6	~1 x 10^7
Tissue	~10 mg	~20 mg

2. Add buffer RL to the sample.
Make sure that reducing agents are added to buffer RL.

	Standard	Large Input
Quantity of Buffer RL	350 µl	600 µl

3. Add ethanol (70 % v/v) to the lysate and mix well by pipetting.

	Standard	Large Input
Quantity of Ethanol	350 µl	600 µl

For the subsequent steps both "Standard" and "Large Input" are the same operation.

4. Take a RNA binding column (green) placed in a collection tube. Load up to 700 µl of the mixture into the RNA binding column and centrifuge ≥10,000 x g for 1 min at room temperature (20-25°C).
For large input, discard the flow-through and repeat this step until no more lysate is available.

5. Add 600 µl of buffer RW1 and centrifuge at ≥ 10,000 x g for 30 seconds at room temperature (20- 25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.

6. Add 700 µl of buffer RW2* and centrifuge at ≥ 10,000 x g for 30 seconds at room temperature (20- 25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.
**Make sure that ethanol is added to buffer RW2.*

7. Centrifuge at full speed for 1 min at room temperature (20-25°C) to remove residual buffer RW2. Transfer MQ RNA binding column to a new 1.5 ml collection tube.

8. Add 50 µl of buffer RE to the center of the membrane of the MQ RNA binding column. Centrifuge at ≥ 10,000 x g for 1 minute at room temperature (20-25°C) in order to elute the purified RNA.

RNA quantification, quality and storage

We recommend to determine the quantity and quality of isolated RNA to ensure best conditions for every downstream application. The easiest way to determine the concentration and purity of isolated RNA is to measure the absorbance at 260 nm and 280 nm with a spectrophotometer. 40 µg of RNA/ml corresponds to 1 O.D. unit measured at 260 nm. For spectrophotometric analysis it is advisable to dilute the sample in a buffered solution, e.g. TE (Tris EDTA) buffer. Due to the DEPC treatment the RE buffer is slightly acidic and can cause a decrease of absorbance values, so it is not recommended to measure RNA absorbance with RE buffer. Pure nucleic acids have an A260/A280 ratio of 2.0 and pure proteins one of 0.6. On that account a ratio value of 1.8-2.0 represents 90-100% pure nucleic acid.
RNA quality can be also assessed by electrophoresis analysis. In optimum case for eukaryotes two distinct bands should appear on the gel – the 28S and 18S (23S and 16S for bacteria) ribosomal RNA bands. Degradation during preparation, handling or storage results in a smear towards lower molecular weight sized RNAs.
To ensure RNA stability keep RNA frozen at -20°C for short-term or -70°C for long-term storage.