MQ Advanced RNA Isolation Kit

Cat No. ARI-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)	20 ml
RNA re-binding buffer (RBD)	8 ml
Elution buffer (RE: RNase free water)	30 ml
DNase I reconstitution solution	1.5 ml
10 x DNase I reaction buffer	150 µl
DNase I (lyophilized)	110 units
MQ RNA filter column (yellow, with collection tubes)	50
MQ RNA binding column (green, with collection tubes)	50
MQ RNA mini-elute column (neutral, with collection tubes)	50
1.5 ml collection tubes	100
2 ml collection tubes	150

Storage

Store the MQ Advanced RNA Isolation kit at room temperature (15-25°C) except MQ RNA mini-elute columns (neutral color) which should immediately be store at 2-8°C after receipt. Storing MQ RNA mini-elute column at room temperature will reduce performance.

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 Advanced 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.

The MQ Advanced RNA Isolation Kit guarantees pure high-quality RNA due to an optimized DNase I treatment in combination with a specifically engineered MQ mini-elute column technology. Unlike in kits of other suppliers the DNase I treatment will take place in liquids and not on top of the column membrane. This increases the DNase I efficiency a lot. Following RNA is bound to the membrane of the MQ mini-elute column that possesses a high RNA binding capacity. A much higher RNA concentration can be reached due to the small column diameter, so that elution volume can be decreased to 10 µl.

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.

2. Second wash buffer RW2

Add 80 ml of 96-100% ethanol to 20 ml RW2 and mix.

3. Lyophilized DNase I

Add 55 µl DNase I reconstitution solution to a tube of lyophilized DNase I.
In order to collect the DNase on the bottom of the vial spin down the powder by using a centrifuge before opening the tube. Add the indicated volume of the DNase I reconstitute solution, mix gently by tapping the tube. Do not vortex DNase! Dissolved DNase I can be stored in aliquoted tubes at -20˚C. We do not recommend to refreeze and thaw the enzyme.

4. RNA re-binding buffer RBD

Add 7 ml of 96-100% ethanol to 8 ml RBD.

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 Advanced 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 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
- Addition of ethanol to buffer RBD
- Reconstitution of DNase I

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. Take a MQ RNA filter column (yellow) placed in a collection tube. Transfer lysate into a MQ RNA filter column and centrifuge at ≥ 10,000 x g for 1 min at room temperature.

4. 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.

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

6. 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.

7. 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.*

8. 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.

9. 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.

10. Add 5 µl of 10 x DNase I reaction buffer to the 50 µl of the eluted sample and mix well by pipetting.

11. Add 1 µl of DNase I enzyme solution* to the mixture, mix thoroughly by pipetting and incubate for 10 minutes at room temperature (20-25°C).
* Prepare DNase I solution before use.

12. Add 250 µl of buffer RBD* to the DNase I treated mixture and mix well by pipetting.
*Make sure that ethanol is added to buffer RBD.

13. Take a MQ RNA mini-elute column (neutral) placed in a collection tube. Apply all of the mixture into the MQ RNA mini-elute column and centrifuge at ≥ 10,000 x g for 1 minute at room temperatur (20-25°C).

14. Apply 700 µl of buffer RW2 into the MQ RNA mini-elute column and centrifuge at ≥ 10,000 x g for 30 seconds at room temperature (20-25°C), discard the flow-through and re-insert the MQ RNA mini-elute column to a new 2 ml collection tube.

15. Centrifuge at full speed for 1 min at room temperature to remove residual buffer RW2. Transfer MQ RNA mini-elute column to a new 1.5 ml collection tube.

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

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise.

Problem	Cause	Solution
No or low RNA concentration	Too small amount of starting material	Increasing of starting material up to the material specific recommended amount
	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Insufficient homogenization or disruption of starting material	Pulverize starting material in liquid nitrogen as indicated to obtain a fine powder.
	Incomplete elution of RNA from spin column membrane	Repeat elution step with a prior warming of the RNase free elution water to 60°C
	Incorrect DNase I reaction mixture	Be sure to comply with the instruction
Problem	**Cause**	**Solution**
Filter column is blocked	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Too small amount of starting material	Increasing of starting material up to the recommended amount
	Insufficient homogenization or disruption of starting material	Complete homogenising of starting material and increasing of centrifugation time
Problem	**Cause**	**Solution**
RNA degradation	RNase contamination	Decontamination of all by user supplied plastics, reagents and work equipment
Problem	**Cause**	**Solution**
DNA contamination	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Incorrect DNase I reaction mixture	Be sure to comply with the instruction

Problem	Cause	Solution
Low A260/ A230 ratio	Acidic buffer or water used for RNA dilution	As DEPC treated water becomes weakly acidic and decreases the absorbance value, please use TE buffer etc.
	Amount of sample material is too high	If the sample amount is too high, impurities could lead to a clogged membrane. Co-purified proteins or DNA can change the OD ratio.
	For cultured cells: Medium was not removed efficiently from cultured cells	Please completely remove the medium from the cell pellet. Residual medium leads to insufficient lysis procedure.
	Incomplete DNase I digest	Increasing of DNase I incubation time
Problem	**Cause**	**Solution**
Suboptimal performance in downstream applications	Salt contamination	Before drying the membrane by centrifugation, please use a new collection tube. In some cases it may be good to repeat washing step with the second wash buffer (RW2). RW2 must have room temperature.
	Incorrect storage of RNA	Keep diluted RNA on ice and store RNA for long term at -70°C or colder.
	Residual ethanol	After washing with buffer RW2, dry the membrane according to the protocol. ① When you remove the column, please make sure that the column is not in touch with the liquid inside the collection tube.. ② Add the elution buffer RE to the center of the membrane. Carryover of Ethanol will affect downstream applications.

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.