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mi-Mag mRNA Isolation Kit

Cat. No mi-CMM050

[50 Reactions]

**This kit is for research purposes only.
Not for use in diagnostic procedures.
For *in vitro* use only.**

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Introduction

This kit contains enough materials for 50 isolations of mRNA directly from 10^7 cells, 100 mg animal tissue or 100 mg plant tissue and is optimized for the use with a magnetic separator. The included protocol can be individually adapted and can be scaled up for mRNA isolation from $10^3 - 10^7$ cells, 10 - 100 mg animal or plant tissue. 40 μ l of Magnetic Bead suspension can bind at least 2 μ g mRNA.

Kit Contents mi-Mag mRNA Isolation

Lysis Buffer	50 ml (store at 4 °C)
Magnetic Beads	2 ml (store at 4 °C)
Suspension Buffer	30 ml
Wash Buffer A	50 ml
Wash Buffer B	100 ml
Elution Buffer	5 ml

Required Equipment

Magnetic separator
Microcentrifuge (13,000 rpm or 12,000 x g)
Homogenizer
Heating Block (70 °C)
Vortexer
Microcentrifuge tubes
Nuclease-free water (pH 7-8) or 10 mM Tris-HCl (pH 8.0)

Kit Storage

All solutions may be stored at room temperature (15-25 °C), with the exception of the Magnetic Beads and the Lysis Buffer, which are best stored at 4 °C.
All buffers should be brought to room temperature before starting.

Precautions

See MSDS on our homepage (www.mymetabion.com).
The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves and safety glasses when using it.

Protocol

Note: **Before starting, please make sure....**

- The material getting in contact with RNA is free of contaminating RNases.

The extreme instability of RNA is mainly due to the ubiquitous presence of enzymes (RNases) which degrade RNA. So decontaminate all equipment following protocols to create a Ribonuclease-free environment.

How to prevent RNase contamination:

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper techniques. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA:

1. Always wear gloves when working with RNA.
2. Sterile, disposable plasticware can safely be considered RNase-free and should be used when possible.
3. Contaminating RNases can be inactivated by baking glassware at 180 °C or higher for several hours. Alternatively, glassware can be soaked in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, drained and autoclaved (necessary to destroy any unreacted DEPC which can otherwise react with other proteins and RNA). DEPC will destroy polycarbonate or polystyrene materials (e.g. electrophoresis tanks), which should instead be decontaminated by soaking in 3% hydrogen peroxide for 10 minutes. Remove peroxide by extensively rinsing with RNase-free water (see below) prior to use.
4. Metal spatulas can quickly be decontaminated by holding in a burner flame for several seconds.
5. It is a good idea to maintain a separate area for RNA work that has its own set of pipettors. This is especially important if your work requires the use of RNase A (e.g. plasmid preps).

Prewash of Magnetic Beads

1. Shake bead suspension vigorously and transfer 40 µl (or up to 120 µl) to a new 1.5 ml microcentrifuge tube.
2. Place the tube in a magnetic rack and separate the beads magnetically.
3. Remove the storage buffer and wash the beads by resuspending in 300 µl of Suspension Buffer.
4. Repeat step 3.
5. Resuspend the Magnetic Beads in 100 µl of Lysis Buffer.

Preparation of Samples

Disrupt and homogenize 1 - 100 mg tissue (fresh or frozen) or 10^7 cells using one of the following methods:

1. **Bead-mill or rotor-stator**
Add 300 μ l of Lysis Buffer to the sample in a 2 ml screw cap tube and homogenize according to the instrument supplier's instructions.
2. **Mortar and Pestle**
Thoroughly grind the sample in liquid nitrogen to obtain a fine powder.
Add 900 μ l of Lysis Buffer to the still frozen powder in the tube.
Reduce the viscosity of the lysate using a syringe with a 21G needle (pass the liquid 10 times through the needle).

Purification Protocol for mRNA from 10^3 - 10^7 cells, 10 - 100 mg animal or plant tissue

1. Spin the lysate at 13,000 rpm (12,000 x g) for 5 - 10 min to pellet the cell debris.
2. Transfer the supernatant into the tube containing the washed Magnetic Beads.
3. Resuspend the beads in the lysate and incubate for 2 - 5 min at 70 °C.
4. Incubate for 7 min at room temperature with occasional gentle mixing.
5. Following incubation, place the tube in a magnetic separator and wait until all the beads have been attracted to the side of the tube.
Aspirate off all of the supernatant and remove the tube from the magnet.
6. Add 900 μ l of Wash Buffer A to the tube.
Gently resuspend and wash the beads with 5 pipetting strokes.
7. Separate the beads magnetically and remove the supernatant.
8. Repeat steps 6 through 7 another two times with 1000 μ l of Wash Buffer B.
9. Add 50 - 100 μ l of Elution Buffer and resuspend the beads.
Incubate the suspension for 2 min at 70 °C with vigorous mixing to facilitate complete mRNA elution.
For a RT-PCR it is possible to use 5 μ l to 10 μ l of the bead suspension to perform the first-strand cDNA synthesis.
10. After the incubation, place the tube in the magnetic separator. Wait until the supernatant is clear (at least 2 min).
Transfer the eluate containing the purified mRNA to a clean RNase free tube.

Regeneration for Re-use

After the mRNA isolation the Magnetic Beads can be reused, if regeneration as follows:

1. Add 500 µl of 0.1 N NaOH, resuspend and incubate for 10 min at room temperature. Magnetically separate and discard supernatant.
2. Repeat step 1.
3. Resuspend in 500 µl of water and incubate for 5 min at 95 °C. Separate magnetically and discard supernatant.
4. Wash twice with 500 µl TE buffer (pH 8).
5. For re-use, start with step 2 of the isolation protocol.

Hints and Troubleshooting

UV Measurements

In some cases there may be some traces of the Magnetic Beads left in the eluate after removal from the tube. Such particles will not interfere with most downstream applications such as PCR but may increase the background in UV measurements.

In such cases, prior to UV analysis, we recommend an additional application of the magnet to the elute for 3 min in order to separate any traces of particles.

For pure mRNA the expected A_{260}/A_{280} ratio is between 1.9 and 2.3. The A_{260} value should be between 0.1 and 1.0 for significant readings.

Low yield

Sample condition

The sample must be placed on ice when working with it.

Insufficient lysis or binding to Magnetic Beads

- Mix samples thoroughly upon addition of the Lysis Buffer.
- It may help to extend the lysis time.
- It may help to lengthen the hybridization time.

Incomplete elution

Verify that the elution temperature was correct and, if necessary, extend the elution time.

Wash Buffer not removed sufficiently

Ensure that as much buffer as possible is removed between the washing steps before proceeding further.

Bead pellet not properly resuspended in elution step

Resuspend the bead pellet in elution buffer until the pellet is homogeneously dispersed.

A_{260}/A_{280} is too low

Protein contamination

- Beads were not sufficiently resuspended during the washing steps.
- If necessary the repeat purification protocol omitting the Lysis Buffer step.

Residual beads in eluate

- Incomplete separation of the Magnetic Beads from the eluate can increase the background of UV measurements.
- Repeat magnetic separation and transfer eluate to a clean tube.
- Residual Magnetic Beads will not affect most downstream applications.



Precipitate in reagent bottle

Bottles stored below room temperature

Warm the reagent bottle in water bath (37° C) to redissolve the precipitate.

Degraded RNA

Old Sample or sample has been repeatedly frozen and thawed.

To reduce the RNase activity in frozen samples, thaw them quickly in a 37° C water bath and then place on ice until use.

RNase contamination

Replace all buffers