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chemagic mRNA T Kit

for general purposes

Kit for the isolation of mRNA from total RNA

Kit Components

Magnetic Beads	2 ml
Binding Buffer 1	55 ml
Wash Buffer 2	55 ml
Elution Buffer 3	5 ml

This kit contains sufficient reagents for the isolation of at least 100 µg mRNA from total RNA and is optimized for use with **chemagic** Magnetic Separators.

Storage Conditions

The **Magnetic Beads** should be stored at 4 °C. Store all buffers at room temperature or 4 °C. If stored at 4 °C, the buffers should be brought to room temperature before use.

Samples and Protocol Adjustments

The included protocol is scaleable for different starting amounts of total RNA. 40 µl of **Magnetic Bead** suspension can bind at least 2 µg mRNA.

UV Measurements

In some cases the inexperienced manual user may find some traces of the magnetic particles in the eluate. Such particles will not interfere with most downstream applications but may increase the background in UV measurements. In such a case, prior to UV analysis, chemagen recommends an additional application of the magnet to the eluate for 2 minutes in order to separate any traces of particles.

Further Questions?

chemagen technical support

Phone +49 (0) 2401 805-501

Mail support@chemagen.de

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Protocol

1. Shake bead suspension vigorously and transfer 80 µl to a new reaction tube.
2. Separate the beads magnetically until the supernatant is clear, remove and wash the beads **twice** with 300 µl **Binding Buffer 1**.
3. Resuspend the **Magnetic Beads** in 300 µl **Binding Buffer 1**.
4. Dissolve 100 µg total RNA in 300 µl water and incubate at 65 °C for two minutes.
5. Add the RNA solution to the bead suspension and mix gently, incubate at 65 °C for two minutes and then for 10 minutes at room temperature with gentle mixing. Following incubation, place the tube in a chemagen Magnetic Separator to draw the beads to the side of the tube. Wait 30 seconds or until the all the beads have been attracted to the magnet. Pipette off the supernatant and then remove the tube from the magnet.
6. Add 400 µl **Wash Buffer 2** to the tube. Gently resuspend the beads with 2 pipetting strokes. Separate the beads magnetically and remove the supernatant.
7. Repeat step 6.
8. Add 50 - 100 µl **Elution Buffer 3**, which has been prewarmed to 70 °C, and then resuspend. Incubate the suspension for 2 minutes.
9. After incubation place the tube in the Magnetic Separator for at least 30 seconds to separate all the beads from solution. Remove the eluate containing the purified mRNA to a clean tube.
10. To increase yield repeat steps 8 and 9.

Regeneration for Re-use

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

1. Add 500 µl of 0.1 N NaOH, resupend and incubate for 10 minutes at room temperature. Magnetically separate and discard supernatant.
2. Repeat step a.
3. Resuspend in 500 µl of water and incubate for 5 minutes at 95 °C. Magnetically separate and discard supernatant.
4. Wash twice with 500 µl TE buffer (pH 7 - 7.5).
5. For reuse start with step 2 of the isolation protocol.

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