



MESSENGER RNA STANDARD ISOLATION KIT

Product No. **MRI-2**
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Product Information

TECHNICAL BULLETIN

Sigma's Messenger RNA Standard Isolation Kit enables the researcher to isolate and purify PolyA⁺ mRNA from cultured cells or small tissue samples in as little as 1-2 hours. The tissue or cells are lysed in detergent (SDS)-based buffer that contains a protein/RNase dehydrator and applied to oligo(dT) cellulose. The isolated mRNA can be used immediately or stored for several months at -70°C. Typical yields reported are 10-85 mg/10⁸ cultured cells or 5-100 mg/g tissue. Yields can vary with the type, condition and growth stage of the cells or tissue used. The mRNA obtained is suitable for applications such as cDNA synthesis, Northern blots, RNase protection, RT-PCR and library construction.

For smaller sample sizes (1 x 10² to 5 x 10⁶ cultured cells or 5-200 mg tissue) the Messenger RNA Micro Isolation Kit, Product No. MRI-1 is recommended.

Reagents Provided

Sufficient for 6 isolations

- Stock Buffer, Product No. S5415
100 ml
- RNase/Protein Degradator, Product No. P5700
2 x 1.25 ml
- Sodium Chloride, 5 M Solution, Product No. S6922
8 ml
- Oligo(dT) Cellulose, Product No. O0633
6 x 75 mg
- Low Salt Wash Buffer, Product No. W3379
3 x 100 ml
- Binding Buffer, Product No. B1286
2 x 110 ml
- Elution Buffer, Product No. E7767
2 x 8 ml
- Sodium Acetate, 2 M Solution, Product No. S5665
4 ml

- Disposable Spin Columns/
2.0 ml Microcentrifuge Tubes, Product No. S7415
6 each
- 1.5 ml Microcentrifuge Tubes,
Product No. M2792
6 each

Equipment and Reagents Required But Not Provided
(Sigma product numbers have been given where appropriate)

- Phosphate-buffered saline (PBS), Product No. D1408
- Sterile 50 ml centrifuge tubes
- Water bath
- Shaking water bath
- Table-top centrifuge
- Absolute ethanol
- Microcentrifuge
- Homogenizer or mortar and pestle
- Disposable plastic syringe (25 ml) fitted with 18-21 gauge needle
- Liquid nitrogen (optional)

Storage

Store the oligo(dT)-cellulose, Product No. O0633 at -20°C. Store all other components at 2-8°C.

Precautions and Disclaimer

Sigma's Messenger RNA Standard Isolation Kit is for laboratory use only. Not for drug, household or other use. Kit contains components which are hazardous. Warning statements are included on the label or in the components section of this bulletin where applicable.

RNA work requires treating glassware or plasticware for possible RNase contamination, unless it is disposable and individually wrapped or specifically recommended for RNA work by the supplier. See Sambrook¹ for a discussion of methods of treating labware to eliminate RNase contamination.

Procedure

A. Preliminary

1. If the Stock Buffer has a white precipitate (SDS), warm briefly at 65°C to redissolve.
2. Warm RNase/Protein Degradar to 45°C and invert until solution is clear. Prepare Lysis Buffer by adding 0.3 ml RNase/Protein Degradar to 15 ml Stock Buffer.

B. Preparation of Tissue-cultured Cells

Use 1×10^7 to 3×10^8 log-phase cells for each mRNA isolation.

1. For monolayer cells, remove nutrient solution and quickly wash cells in 4°C phosphate-buffered saline (PBS). **Note: Do not include trypsin; it may exhibit RNase activity.** Remove PBS and add 15 ml lysis buffer. Transfer cell suspension to a suitable homogenizer tube. Continue with Step D-1a.
2. For cell suspension, centrifuge and remove supernatant growth medium. Quickly wash cells once with an appropriate volume of 4°C PBS. Centrifuge to pellet the cells, remove PBS and resuspend cells in 15 ml lysis buffer. Transfer cell suspension to a suitable homogenizer tube. Continue with Step D-1a.

C. Preparation of Tissue

1. Excise 0.4-1.0 g tissue and transfer to a suitable homogenizer tube containing 15 ml lysis buffer. Proceed with Step D-1a. Tissue may also be homogenized in liquid nitrogen using a mortar and pestle (see Step D-1b).
2. If tissue is not to be immediately used, freeze it in liquid nitrogen and store at -70°C. It can then be stored at -70°C for several months.

D. mRNA Isolation

- 1a. Homogenizer: Homogenize the tissue sample or cell suspension in a suitable homogenizer with 10-12 up and down strokes, or until the viscosity resembles that of the lysis buffer. Transfer lysate to a 50 ml sterile centrifuge tube and pass lysate through an 18-21 gauge needle fitted onto a sterile plastic syringe 3-5 times. The high molecular weight DNA is sheared in this step resulting in a cleaner mRNA preparation. Some tissue and cell suspension samples may be suitable for lysing and homogenizing by the syringe method alone. Proceed with Step 2.
- 1b. Mortar and pestle: Alternatively, frozen tissue may be homogenized under liquid nitrogen in a mortar and pestle. After grinding is complete transfer the suspension of tissue and liquid nitrogen to a sterile 50 ml centrifuge tube and allow liquid nitrogen to evaporate. Add 15 ml lysis buffer and mix. Transfer lysate to a sterile plastic syringe fitted with an 18-21 gauge needle and pass lysate through 3-5 times. Proceed with Step 2.
2. Incubate lysate for 60 minutes at 45°C in a slowly shaking water bath. During this step, proteins, including RNases are digested. If, after incubation, insoluble material is still visible, centrifuge at 4000 x g for 5 minutes at room temperature and transfer supernatant to a new centrifuge tube.
3. Adjust the NaCl concentration in the lysate by adding 950 µl of sodium chloride, 5 M solution to the entire volume of lysate (approx. 15 ml) and mix thoroughly.
4. Pass the lysate through an 18-21 gauge needle fitted onto a sterile plastic syringe 3-4 times to shear the DNA.
5. Remove a vial of oligo(dT)-cellulose from the freezer, and pour it into the lysate. Seal the tube and allow the oligo(dT)-cellulose to swell for 2 minutes. The oligo(dT)-cellulose should disperse readily. If the oligo(dT)-cellulose does not disperse within 5 minutes, manually disperse with a pipette tip.
6. Rock the tube gently at room temperature for 15-60 minutes. A rotary shaker will facilitate this process.

7. Centrifuge the suspension at 4000 x g in an appropriate centrifuge for 4-8 minutes.
8. Carefully remove supernatant and gently resuspend the oligo(dT)-cellulose in 20 ml binding buffer. Mix well to wash and remove binding buffer by centrifugation. Wash again with 10 ml binding buffer and remove by centrifugation. Repeat at least 3 times until buffer is no longer cloudy. See Note 2.
9. After the last wash suspend the oligo(dT)-cellulose in 10 ml low salt wash buffer. Repeat this wash step until buffer is no longer cloudy. When washing is complete, suspend oligo(dT)-cellulose in a volume of low salt wash buffer not to exceed 800 μ l.
10. Transfer the suspension to a disposable spin column/2.0 ml microcentrifuge tube. Spin in a microcentrifuge at 5000 x g for 10 seconds at room temperature. Remove the spin column and discard the eluate in the microcentrifuge tube. Replace spin column, fill to top with low salt wash buffer and mix the oligo(dT)-cellulose until suspended with a sterile pipet tip. Centrifuge as above. Discard eluate and repeat wash step at least three times or until the A_{260} of the eluate is 0.05. See Note 3.
11. Transfer the spin column to a fresh microcentrifuge tube (supplied). To elute the mRNA, add 200 μ l of elution buffer and stir the oligo(dT)-cellulose in the buffer with a sterile pipette tip. Spin in a microcentrifuge at 5000 x g for 10 seconds at room temperature. Wash oligo(dT)-cellulose again with 200 μ l of elution buffer as above.
12. Remove the spin column from the tube. The microcentrifuge tube should now contain 400 μ l of mRNA in elution buffer. If the volume is smaller, centrifuge the unit again for one minute.
12. To the eluate in the microcentrifuge tube add 0.15 volumes of sodium acetate, 2 M solution and 2.5 volumes of absolute ethanol (100%), mix thoroughly and place in a -70°C freezer until solid. Spin in a microcentrifuge for 15 minutes at maximum speed (16,000 x g). Remove and discard ethanol. Dissolve the mRNA in 20-50 μ l of elution buffer, depending on the desired concentration.

Notes

1. After the 45°C incubation, all manipulations except the final precipitation of mRNA should be carried out at room temperature. This avoids precipitation of the SDS. If a microcentrifuge rotor was recently used at 4°C, it should be warmed first.
2. It may be difficult to remove all the wash buffer without disturbing the oligo(dT)-cellulose pellet. Usually approximately 80% can be safely removed. If this is the case, the next addition of wash buffer should only use 80% of the volume recommended in the procedure. Repeat washing until all the SDS is removed. To test if SDS is present in the wash, shake the tube briefly. If bubbles form, SDS is present. Continue washes until no bubbles are seen.
3. In Step 10, if some oligo(dT)-cellulose remains in the tube after transfer, add a few μ l of binding buffer to wash out the tube and transfer the oligo(dT)-cellulose suspension to the spin column.
4. If the mRNA is to be used on completion of this isolation, it should be kept at -70°C for at least 15 minutes for optimum precipitation, although 16-72 hours (overnight or over a weekend) is common.
5. The mRNA eluted in elution buffer (10 mM Tris, pH 7.5) is suitable for cDNA applications. It can also be precipitated and dissolved in sterile, RNase-free water.

References

1. Sambrook, J., *et al.* Molecular Cloning: A Laboratory Manual Second Edition, Cold Spring Harbor, (1989), p. 7.2.

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