mRNA Magnetic Purification Kit

Product description

mRNA Magnetic Purification Kit is designed for purifying mRNA from total RNA or in vitro transcription, which uses monodisperse superparamagnetic beads that covalently couple oligomeric dT sequences on its surface. High purity mRNA is achieved by base pairing between the oligo-DT sequence and the poly(A) tail of the mRNA. The dT sequence combined with mRNA can be directly used as the first chain reaction primer to synthesize cDNA, or mRNA can be eluted from the beads for various downstream molecular experiments, such as RT-PCR, RPA (ribonuclease protection assay), library construction, Northern Blot analysis, next-generation sequencing, etc.

Composition and storage conditions

Components \ Size	2 mL	5 mL
Oligo (dT) 25 beads (10 mg/mL)	2*1 mL	5*1 mL
Binding Buffer	5 mL	3*5 mL
Washing Buffer	5 mL	3*5 mL
Tris-HCl Buffer	1 mL	3*1 mL
RNase-free water	2*1 mL	5*1 mL
Store at 4 °C, no freezing!		

Experimental operation

Required materials: magnetic stand, water bath, Rnase-free centrifuge tube, RNase-free tip, mixing device, etc.

Protocol

Take the purification of mRNA from total RNA(60-80 μg) as an example, please adjust the dosage proportionally according to the actual sample.

1. Wash Oligo (dT) 25 beads

a) Resuspend the Oligo (dT) 25 beads in the reagent bottle (vortex>2 min or manually shake to mix the beads thoroughly).

b) Pipette 100 µL beads suspension to RNase-free centrifuge tubes, place the tube on the magnetic stand for 1 min, discard the supernatant.
c) Add the same volume of Binding Buffer and resuspend the beads. Place the tube on the magnetic stand for 1 min and discard the supernatant.
d) Add 100 µL Binding Buffer to resuspend the bead.

2. Sample preparation

a) Adjust the volume of the total RNA sample (60-80 µg) to 100 µL with Rnase-free water or Tris-HCl Buffer, and then add another 100 µL of Binding Buffer into the sample.
b) Heat the 200 µL total RNA solution at 65 °C for 2-5 min (disruption of RNA secondary structure), then rapidly transfer the sample to ice.

3. Binding

a) Add the 200 µL total RNA solution to 100 µL washed beads (1 mg of bead is required for every 60-80 µg of total RNA), mixing well by repeated pipetting.
b) Shake gently at room temperature for 5 min to bind the beads and mRNA.

4. Washing

a) Place the tube on a magnetic stand for 1-2 min, and carefully discard the supernatant.
b) Remove the tube from magnetic stand, add 200 µL Washing Buffe and mix carefully by several pipettings.
c) Place the tube on the magnetic stand for 1-2 min and discard the supernatant.
d) Repeat steps b and c again to complete the washing.
e) If it is no need to elute the mRNA from the beads, the beads should be washed again before operation by using the same buffer from the downstream experiment.

5. Elution (optional)

a) Remove the tube from the magnetic stand and resuspend the beads by adding 10-20 µL of Rnase-free water or Tris-HCl Buffer.
b) Heat at 75-80°C for 2 min, then stand on the magnetic stand for 1-2 min, and quickly collect the supernatant containing mRNA to a new RNase-free centrifuge tube.
c) It is recommended that eluted mRNA samples should be used for downstream experiments as soon as possible. Alternatively, an RNase inhibitor is added to the eluted mRNA and stored at -20°C for more than a month or -80°C for several months

Notes

1. The experimental process should strictly ensure that there is no RNase or nucleic acid contamination, it is recommended to change gloves frequently to reduce the possibility of RNase contamination, and only use RNase-free disposable tips and centrifuge tubes for experiments.
2. The magnetic beads must be warmed to room temperature and mixed thoroughly before use, otherwise the recovery efficiency may be affected. Never store the beads at -20°C, because low temperature will damage the bead structure.

3. The beads must always be in liquid to prevent drying of magnetic beads resulting in reduced extraction efficiency.
4. The total RNA with good integrity make better purification effect. Ensure the RIN value ≥ 7.0.
5. When performing small-volume purification experiments, the eluted mRNA supernatant should be collected as soon as possible, since the mRNA may rebind to the beads due to rapid temperature changes in small volume. In addition, remove all washing buffer as much as possible during washing process.
6. This product is for scientific use only.

Troubleshooting

1. mRNA yields are low

Probable Causes	Corresponding Suggestion
The ratio of beads to sample is too low	Appropriately increase the amount of magnetic beads, or reduce the sample volume and increase the sample concentration.
Binding time is too short	Increase the binding time appropriately to 10-15 min.
Inadequate elution	Appropriately increase the volume of the eluent, the elution time and the elution temperature; Or repeat the elution twice, collecting and mixing the two elution products.
The mRNA is expressed at a low level	The selected cells or tissues have low levels of mRNA expression. It is recommended to select samples with a suitable expression period.

2. The bead sorting is poor

Probable Causes	Corresponding Suggestion
Beads clump togather	The negative charge of oligonucleotide dT on the magnetic bead makes the bead surface charged, the interaction between proteins and beads, as well as the electrostatic interaction between the beads and the tube wall caused by repeated magnetic treatment, will cause magnetic bead agglomeration. These electrostatic interactions can be reduced by adding 0.05% Tween 20 to binding buffers or washing buffers. Handling too many samples can also lead to beads aggregation, consider reducing sample volume.
The solution is too viscous	The presence of long strands of DNA in the sample can make the solution viscous. The solution can be repeatedly passed through a syringe with a 21 G injection needle to make the sample more homogeneous and reduce the viscosity of the liquid. DNase I treatment can also be added to remove genomic DNA.
The bead separation time is too short	Appropriately extend the time for the beads on the magnetic stand (2-5 min).

3. mRNA contamination

Probable Causes	Corresponding Suggestion
rRNA contamination	rRNA can be effectively cleared by purifying mRNA again. The used beads are washed twice with the washing buffer, the eluted mRNA is diluted with the binding buffer. Then mix the

	beads and eluted mRNA for 3-5 min at room temperature, and repeat the bead separation steps again.
DNA contamination	DNA residue may result from incomplete sample washing or incomplete removal of washing buffer after the binding step. Remove washing solution as much as possible prior to elution, especially for small volume samples. In addition, a high ratio of samples to beads may also be doped with DNA.

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