Oligo dT Magnetic Particles

DESCRIPTION

Oligo dT Magnetic Particles are covalently coupled to Oligo (dT) on the surface, which can complement and pair with Poly A on the tail of eukaryotic mRNA. It can efficiently isolate complete and high-purity mRNA from total eukaryotic RNA or directly from animal and plant tissues or cell lysates. The isolated mRNA can be used in a variety of molecular biology experiments, such as RT-PCR, solid-phase cDNA library construction, RACE, Northern and so on.

PRODUCT INFORMATION

Type	Oligo dT Magnetic Particles
Diameter	2.8 um
Concentration	5 mg/mL
Binding Ability	1-2 ug/mg Magnetic Particles
Size	2 ml
Buffer	1×PBS，0.1%（v/v）proclin-300
Storage	2~8℃

OPERATING PROCEDURES

1. Preparation

1.1 Binding buffer: 20 mM Tris-HCl (pH 7.5), 1.0 M LiCl, 2 mM EDTA.

1.2 Lysis/binding buffer: 100 mM Tris-HCl (pH 7.5), 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mMDTT.

1.3 Washing solution①: 10 mM Tris-HCl (pH 7.5), 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS.

1.4 Washing solution ②: 10 mM Tris-HCl (pH 7.5), 0.15 M LiCl, 1 mM EDTA.

1.5 Magnetic separator, vortex oscillator, rotary mixer, pipette, 1.5mL centrifuge tube.

2. Clean Oligo(dT) magnetic beads

2.1. Place the magnetic bead bottle on the vortex shaker for 20 s, and resuspend the magnetic bead by shaking. Use a pipette to transfer the required volume of magnetic beads to a new centrifuge tube. Add the same volume of binding buffer and resuspend the magnetic beads.

2.2. Place the centrifuge tube on the magnetic separator and let it stand for 1 min. Use a pipette to remove the supernatant and remove the centrifuge tube from the magnetic separator.

2.3. Add the same volume of binding buffer as the initial volume and set aside.

Note, if you purify mRNA from total RNA, add half of the initial volume, that is, magnetic beads are concentrated to 10 mg/mL.

3. Purification of mRNA from total RNA

3.1 Purify mRNA from 75μg total RNA. Adjust the volume of 75μg total RNA to 100μL with DEPC water.

3.2 Add the same volume of binding buffer (100μL).

3.3 Heat at 65°C for 2 minutes to open the secondary structure, and then quickly transfer to ice. Add 200μL of total RNA solution to 100μL of washed magnetic beads. That is, every 75μg of total RNA is washed with 1mg and dissolved in 100μL of binding buffer magnetic beads. Mix well by pipetting.

3.4 Incubate with rotation for 10 minutes at room temperature.

3.5 Magnetic separation for 1 min, carefully remove the supernatant. Remove the centrifuge tube from the magnetic separator.

3.6 Add 200μL of washing solution ②, carefully pipetting and mixing.

3.7 Magnetic separation for 1 min, carefully remove the supernatant. Remove the centrifuge tube from the magnetic separator.

3.8 Repeat washing once (3.6-3.7) for a total of two washings.

4. Isolate mRNA from cell cleavage

4.1 Wash the cell suspension with PBS and centrifuge to obtain a cell pellet. The cell

pellet can be used immediately, or it can be frozen in liquid nitrogen and stored at -80°C for later use.

4.2 Add 1.0 mL of Lysis/Binding Buffer to the cell pellet (1-4×106 cells). Repeated pipetting several times to ensure complete lysis. The DNA released during the lysis process will cause the solution to become viscous, indicating that the lysis is complete.

4.3 Reduce the viscosity through the DNA shearing step. The lysate is processed 3 times through a 21 gauge needle using a 1-2 mL syringe. Repeated shearing may cause foaming of the lysate, but foaming should not affect mRNA yield. The foam can be reduced by 30s centrifugation.

4.4 The lysate can be used for mRNA isolation immediately, or it can be frozen and stored at -80°C for later use.

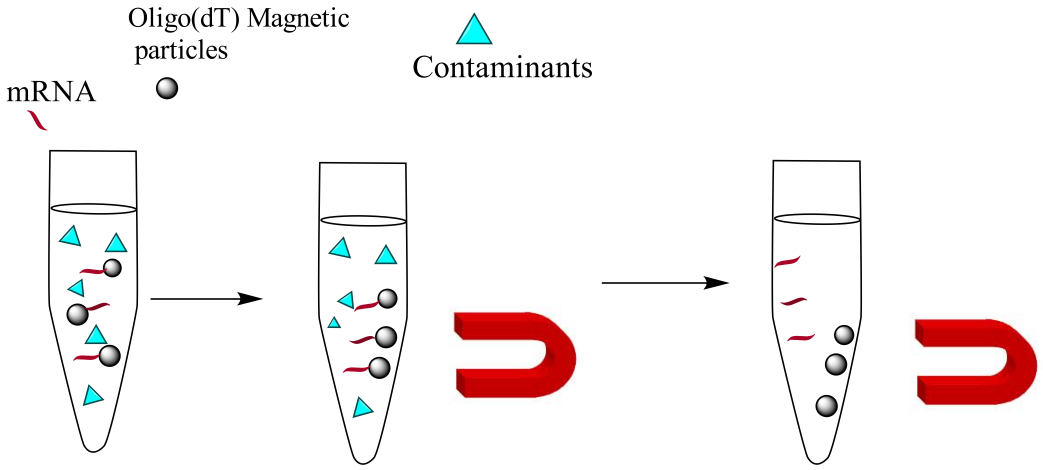
4.5 Magnetically remove the washed Oligo (dT) magnetic beads to remove the supernatant. Add lysate and mix well.

4.6 Rotate and mix at room temperature for 5 minutes to combine. If the solution is viscous, increase the binding time.

4.7 Put the centrifuge tube on the magnetic stand for 1-2 minutes, and remove the supernatant.

4.8 Wash the magnetic beads twice: wash once with 1 mL washing buffer ①, and then wash once with 1 mL washing buffer ②.

Purification of mRNA with Oligo (dT) Magnetic Particles Schematic diagram



Oligo (dT) Magnetic Particle are designed for the rapid isolation of highly purified, intact mRNA from eukaryotic total RNA. It relies on base pairing between the poly-A tail of mRNA and the oligo dT sequences bound to the surface of the beads.

Note

1. In order to reduce the loss of magnetic beads, the time of each magnetic separation should be no less than 1 min.

2. Avoid freezing, centrifugation and other operations during storage of the magnetic bead suspension.

3. It is recommended that the extracted mRNA be used for RT-PCR immediately. If storage is required, it is recommended to add an RNase inhibitor to the eluent to elute the mRNA from the magnetic beads and freeze it.

4. All buffers and consumables used for mRNA extraction should be RNase-free.

5. A mammalian cell generally has about 10-30 pg RNA, of which mRNA accounts for about 1-5%. .

6. Do not dry the magnetic beads for a long time, so as not to cause irreversible magnetic bead aggregation and reduce the efficiency of nucleic acid elution.

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