Oligo dT Magnetic Particles, 0.2 um

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
Concentration	5 mg/mL
Binding Ability	~10 ug mRNA/mg Magnetic Particles
Size	2 ml, 5 ml
Buffer	0.1 M Tris-HCl，pH 7.5, 20 mM EDTA，0.1%（v/v）Tween -20，0.1%（w/v）NaN3
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 mM DTT.

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.

1.6 Sample preparation

1) Animal/plant tissue

a. Quickly and completely homogenize the required amount of plant or animal tissue in liquid nitrogen.

b. Collect the frozen powder and transfer to a fresh tube. Add 1 mL of binding buffer per 100 mg of tissue, mix well, and rotate and mix for 5 minutes at room temperature.

c. Reduce the viscosity of the solution by using a needle to cut the DNA through several times of inhalation and aspiration.

d. Centrifuge at 14,000 rpm for 5 min at room temperature, and transfer all supernatant to a fresh EP tube. The supernatant can be purified for mRNA or stored at -80° C for use.

2) Cell suspension

a. Centrifuge at 4,000 rpm for 5 min at room temperature to pellet the cells and discard the supernatant. Add 1ml binding buffer for every 5-10x106 animal or plant cells.

b. Dissolve the cells by pipetting several times until the solution becomes viscous. Reduce the viscosity of the solution by cutting the DNA with a needle through several aspirations and aspirations.

c. Centrifuge at 14,000 rpm for 5 min at room temperature and transfer all supernatant to a fresh EP tube. The supernatant can be purified for mRNA or stored at -80° C for use.

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 mRSample preparationNA 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

Take purification of 75ug Total RNA as an example

a. Take 100ul containing 75ug Total RNA and mix with 100ul binding buffer,

b. Incubate at 65° C for 2 minutes to open the RNA secondary structure, and immediately place it on ice after the end.

c. Rotate and mix the above mixture at room temperature for 3-5 minutes.

d. Magnetic separation, let it stand for 2min, and remove the supernatant.

e. Wash the magnetic beads with 1ml Washing Solution I and 1ml Washing Solution II at room temperature to remove possible contaminants.

f. Add 10-20μl 10mM Tris-HCl, incubate at 75° C-80° C for 2 minutes, and then quickly transfer the supernatant containing mRNA to a new RNase-free EP tube.

4. Isolate mRNA

a. Prepare animal/plant tissue lysates or prepare lysates from cultured cells and cell suspensions.

b. Process the magnetic beads in accordance with "Clean Oligo(dT) magnetic beads".

c. Mix the lysate with magnetic beads, and rotate and mix for 3-5 minutes at room temperature.

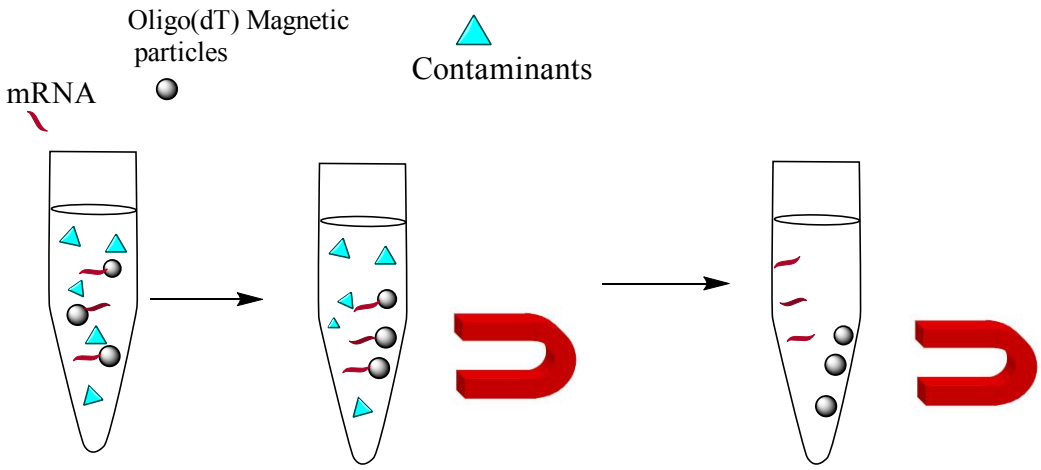
d. Magnetic separation, let it stand for 2min, and remove the supernatant.

e. Wash the magnetic beads with 1ml Washing Solution I and 1ml Washing Solution II at room temperature to remove possible contaminants.

f. If the mRNA is isolated for enzymatic downstream applications (such as solid-phase cDNA synthesis), Washing Solution II (500μL) is washed once, and then washed with enzyme buffer once, which can be used for downstream applications.

If eluting mRNA from magnetic beads, wash with Washing Solution II and add 10-20μl 10mM Tris-HCl, incubate at 75° C-80° C for 2 minutes, and then quickly transfer the supernatant containing mRNA to a new RNase-free EP tube.

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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