Oligo(dT) Magnetic Particles
PRODUCT DATA SHEET

Oligo(dT) Magnetic Particles

Description

Oligo (dT) magnetic beads, good dispersion, fast magnetic response, good hydrophilicity. The covalently coupled Oligo (dT) on the surface of the magnetic beads can be paired with the complementary Poly A of the eukaryotic mRNA tail, which can efficiently separate complete and high-purity mRNA from the 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: RT-PCR, solid phase cDNA library construction, RACE, Northern, etc.

For custom sizes, formulations or bulk quantities please contact our customer service department.

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Characteristics

Oligo(dT) coupling quantity: ~ 400 pmol/mg magnetic beads

mRNA binding amount: 1-2 μg/mg magnetic beads

Concentration: 5 mg/ml

Particle size: 2.8 μm

Surface: Oligo(dT)$_{30}$

Density: 1.4 g/cm^3

Material: Iron oxide

Storage solution: 1 × PBS, 0.01% Tween-20, 0.1% proclin-300

Store: Storage at 2 - 8°C

Quality guarantee period: 24 months

Operation process

1. Prepare before use

1.1 Buffer: The following are commonly used buffer components, users can adjust the buffer formula according to needs, such as SSC, etc.

Binding buffer	10 mM Tris-HCl (pH 7.5), 1.0 M NaCl, 1 mM EDTA
Cracking/binding buffer	100 mM Tris-HCl (pH 7.5), 500 mM NaCl, 10 mM EDTA, 1% SDS, 5 mM DTT
Washing solution ①	10 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA, 0.1% SDS
Washing solution ②	10 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1 mM EDTA
Eluate	Nuclease-Free Water

Note: All reagents need to be prepared with DEPC purified water, balance to room temperature during use, if there is precipitation, can be preheated at 37°C for 10 min.

1.2 RNase-free 1.5 mL centrifuge tube

1.3 Magnetic separator

1.4 Vortex oscillator

1.5 Rotary mixer

1.6 Pipette and suction head

2. Wash

2.1. Place the magnetic bead bottle on the vortex oscillator for 20 s and oscillate to fully re-suspend the magnetic bead. Use a pipette to remove the desired volume of magnetic beads into the new centrifuge tube. The same volume of binding buffer is added to re-suspend the magnetic beads.

2.2. Place the centrifuge tube on the magnetic separator, let it stand for 1 min (this operation is referred to as magnetic separation later), use the pipette to absorb the supernatant, and remove the centrifuge tube from the magnetic separator.

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

Note, if mRNA is purified from total RNA, add half of the initial volume, i.e. magnetic bead concentration to 10 mg/mL.

3. Purification of mRNA from total RNA

3.1 For example, mRNA is purified from 100 μg total RNA. The total RNA volume of 100 μg was adjusted to 100 μL with DEPC water.

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

3.3 Heat at 65°C for 2 min to open the secondary structure and then quickly transfer to ice.

200 μL total RNA solution was added to 100 μL washed magnetic beads. That is, 1 mg per 100 μg total RNA is used after washing and dissolved in 100 μL binding buffer magnetic beads (step 2). Blow and mix well.

3.4 Incubate at room temperature for 10 min.

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

3.6 Add 200 µL washing liquid ②, carefully blow and mix well.

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

3.8 Repeat washing once (3.6-3.7), and wash twice in total.

Note: After washing, the magnetic beads should be completely rehung, and the superbuff should be removed as far as possible after the second washing.

3.9 According to the follow-up experiment, choose one of the following:

1) If it is not necessary to eluate the mRNA from the magnetic beads, the magnetic beads need to be washed again with the enzyme buffer of the downstream experiment.

2) If the mRNA needs to be eluted from the magnetic bead: carefully remove the clean washing buffer ② (be careful not to absorb the magnetic bead), then add 10-20 µL enzym-free water or 10 mM Tris-HCl (pH 7.5), blow and mix thoroughly, incubate at room temperature for 2 min. The tubes were then placed on a magnetic rack and the supernatant containing mRNA was transferred to the new RNase-free tubes.

Note: It can be heated at 65°C-75°C to improve the elution efficiency.

4. Isolation of mRNA from cell cleavage

4.1 Cell suspension was washed with PBS and cell precipitation was obtained by centrifugation. Cell precipitation can be used immediately or frozen in liquid nitrogen and stored at -80°C for later use.

4.2 Add 1 mL lysis/binding buffer to cell precipitation (1-4 × 10^6 cells). Blow it several times to ensure complete cracking. The DNA released during cleavage causes the solution to become sticky, indicating that cleavage is complete.

4.3 Viscosity reduction by DNA shear step. The lysate was treated three times with 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 centrifugation for 30 s.

4.4 The lysate can be immediately used for mRNA separation or frozen and stored at -80°C for future use.

4.5 Remove the supernatant from the cleaned ® Oligo(dT) beads by magnetic absorption. Add the lysate and mix well.

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

4.7 Place the centrifugal tube on the magnetic rack for 1-2 min and remove the supernatant.

4.8 Magnetic bead cleaning twice: wash once with 1 mL washing buffer ①, and then wash once with 1 mL washing buffer ②.

Note: Washing requires the magnetic beads to be completely re-suspended and the supernant to be completely removed between washing steps.

4.9 According to the follow-up experiment, choose one of the following:

1) If the mRNA does not need to be eluted from the magnetic bead (such as for subsequent solid phase cDNA synthesis), wash it again with detergent buffer ② (500 µL), and then wash it with enzyme buffer used in the downstream experiment.

2) Eluting mRNA from magnetic beads: Remove clean wash buffer ②, then add 10-20 µL of enzyme-free water or 10 mM of Tris-HCl (pH 7.5). Incubate at 65°C to 75°C for 2 min, then place the centrifuge tube on a magnetic rack and quickly transfer the mRNA containing supernatant into the new RNase-free centrifuge tube.

Note: Depending on the abundance of mRNA, the final yield may vary between different tissues/cells. A mammalian cell typically contains about 10-30 pg RNA, of which mRNA accounts for about 1-5%.

Notes

1. Avoid freezing magnetic beads.

2. In order to reduce the loss of magnetic beads, the time of each magnetic separation should not be less than 1 min.

3. It is recommended that the extracted mRNA be immediately used for RT-PCR. If preservation is required, it is recommended to add an RNase inhibitor to the eluent, wash the mRNA off the bead and freeze it.

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

5. Before removing the magnetic bead from the magnetic bead storage tube, the magnetic bead should be fully shaken and re-suspended evenly.

6. If the residual amount of purified rRNA is too high for downstream application, a second round of mRNA purification can be performed.

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7. It is recommended to use good quality pipette suction and reaction tube to avoid losses caused by adhesion to magnetic beads and solution.

8. This product is for research use only.

Ordering Information

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