APExBIO
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Protocol
Cat. No. K1306

# Oligo (dT) 25 Beads

## Product description

The mRNA only accounts for 1%-5% of the total RNA in eukaryotic cells, and efficient purification of mRNA is crucial for subsequent experiments. Oligo (dT) 25 Beads are specially designed for the purification of eukaryotic mRNA, which uses monodisperse superparamagnetic beads that covalently couple oligomeric dT sequences on its surface. By base pairing between the oligo dT sequence and the poly(A) tail of the mRNA, high-purity intact mRNA can be quickly separated from eukaryotic total RNA or cells, animal and plant tissues.

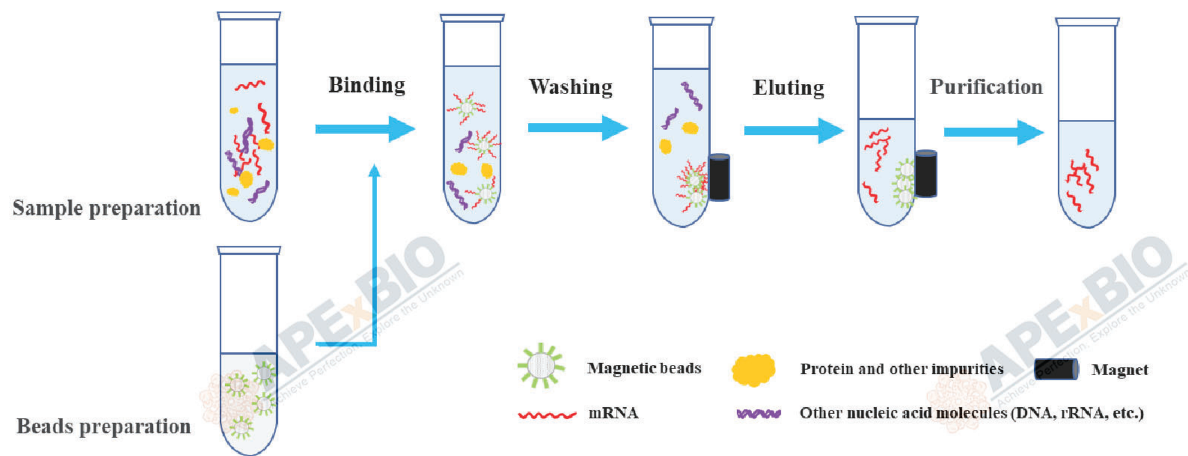
The dT sequence combined with mRNA can be directly used as the first chain reaction primer to synthesize cDNA, or mRNA can be eluted from the beads for various downstream molecular experiments, such as RT-PCR, RPA (ribonuclease protection assay), library construction, Northern Blot analysis, next-generation sequencing, etc.

## Composition and storage conditions

| Components \ Size | 1 mL | 5*1 mL |
|---|---|---|
| Oligo (dT) 25 Beads (10 mg/mL) | 1 mL(10 mg) | 5*1 mL(50 mg) |
| Store at 4 °C for 12-18 months, no freezing! | | |

## Experimental operation

This product uses the base pairing between the oligonucleotide dT sequence on the magnetic beads and the Poly(A) tail of the mRNA to grab the mRNA in the sample, and complete the magnetic bead sorting by magnetic adsorption. The whole operation is simple and fast, mRNA can be efficiently purified from crude samples such as lysed tissues or cells, total RNA, and in vitro transcription products.




## Experiment preparation

1. Required materials: magnetic stand, water bath, Rnase-free centrifuge tube, RNase-free tip, mixing device, etc.
2. Solutions preparation (all reagents need to be prepared by using RNase free $H_2O$):
   Binding Buffer: 20 mM Tris-HCl, 1.0 M LiCl, 2 mM EDTA (pH 7.5)
   Lysis/Binding Buffer: 100 mM Tris-HCl, 500 mM LiCl, 10 mM EDTA, 1% LiDS, 5 mM DTT (pH 7.5)
   Washing Buffer(A): 10 mM Tris-HCl, 0.15 M LiCl, 1 mM EDTA, 0.1% LiDS (pH 7.5)
   Washing Buffer(B): 10 mM Tris-HCl, 0.15 M LiCl, 1 mM EDTA (pH 7.5)
3. 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 the appropriate volume of 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 the Binding Buffer equal to the volume of the initial bead solution to resuspend the bead.

## Protocol

### I Purification of mRNA from total RNA

1. Sample preparation

a) Adjust the volume of the total RNA sample (60-80 μg) to 100 μL with RNase free $H_2O$ or 10 mM Tris-HCl (pH 7.5), 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.

2. 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.

3. 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(B) 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.

4. Elution (optional)

a) Remove the tube from the magnetic stand and resuspend the beads by adding 10-20 μL of RNase free $H_2O$ or 10 mM Tris-HCl (pH 7.5).
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

**II Purification of mRNA from tissue or cell crude lysates**

1. Sample preparation

- Tissue lysates

a) Grind fresh frozen animal tissue (20-50 mg) or plant tissue (100 mg) into powder in liquid nitrogen.
b) Rapidly transfer the tissue powder to a centrifuge tube containing 1 mL of Lysis/Binding Buffer and vortex for 2 min to fully lyse the powder.
c) Centrifuge the lysates for 60 s to remove insoluble debris.

- Cell lysates

a) Wash the cell suspension with PBS and centrifuge to obtain the cell pellet. The cell pellet can be used immediately or stored at -80 °C after freezing with liquid nitrogen.
b) Add 1 mL of Lysis/Binding Buffer to the cell pellet (1-4×$10^6$ cells) and pipette several times to ensure complete cell lysis.

2. Binding

a) Place the washed beads on the magnetic stand and remove the supernatant.
b) Add the lysates into the beads, gently shake and mix for 5 min at room temperature for binding (if the solution is viscous, the binding time can be increased appropriately).

3. Washing

a) Place the tube on the magnetic stand for 1-2 min and carefully discard the supernatant.
b) Wash twice: Wash once with 1 mL Washing Buffe(A) and again with 1 mL Washing Buffe(B)
c) 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.

4. Elution (optional)

a) Remove Washing Buffe(B), add 10-20 μL RNase free $H_2O$ or 10 mM Tris-HCl (pH 7.5) and resuspend the beads.
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 crude lysates can make the solution viscous. The crude lysates 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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