Enriching Beads® Oligo (dT) Magnetic Beads for mRNA Purification

Isolate the mRNA transcriptome in 15 minutes

User Guidance

Enriching Biotechnology

Rev. 1.0 | October 25th . 2018

Why choose Enriching Beads® for mRNA Isolation

- ○ Monosized beads for rapid and efficient separations
- ○ Uniform size for superior reproducibility
- ○ Easy handling, and no sample loss
- ○ Simple operation by magnet

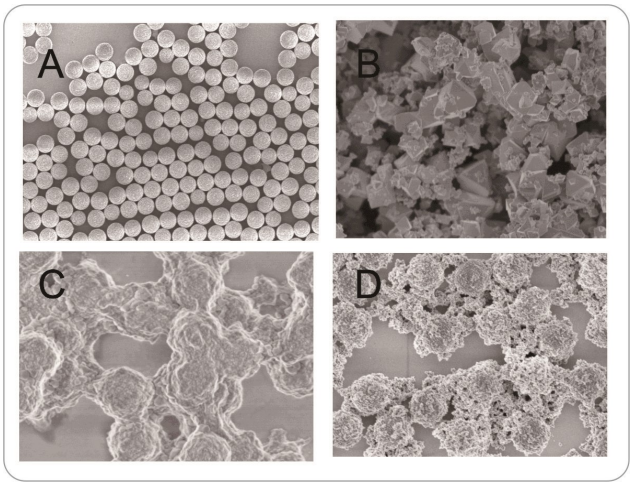

Figure 1: Enriching Beads® Oligo (dT) magnetic beads (A) are monosized, 1 µm magnetic beads with a large, well-defined surface area, providing highly reproducible results. The random sizes and surface areas of magnetic particles from other supplier (B-D) could compromise the reproducibility of your assay results.

Magnetic separation technology

The monodisperse and uniform Enriching Beads® Oligo (dT) magnetic beads provide optimal accessibility and highly reproducible reaction kinetics, ensuring rapid and efficient binding of mRNA molecules under conditions causing minimal stress in 15min. Chemical agglutination and nonspecific binding are negligible with Enriching Beads® compared to irregularly shaped magnetic particles (Figure 1).

The Enriching Beads® Oligo (dT) magnetic beads disperse easily and are handled like a liquid. They exhibit no bead-to-bead magnetic attraction. Due to their superparamagnetic properties, they migrate to the magnet only when placed in a magnetic field. When the magnetic field is removed, the magnetic beads immediately lose all their magnetic remanence and are easily resuspended.

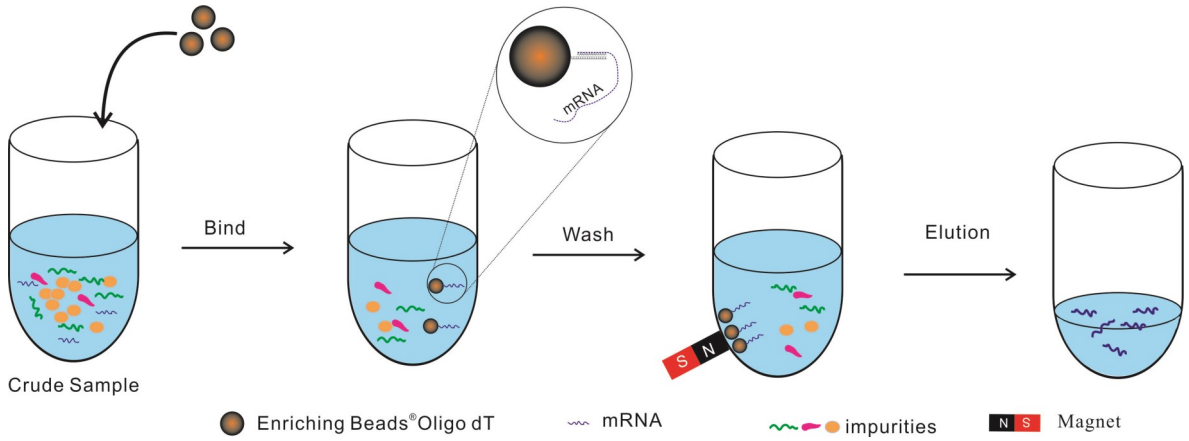

Figure 2: Enriching Beads® Oligo (dT) magnetic beads for mRNA isolation in 15min

Introduction

Enriching Beads® Oligo (dT) magnetic beads (MPS100/Oligo dT) easily isolates and extracts valuable mRNA from a variety of sources (eukaryotic total RNA or directly from crude extractsof cells, animal and plant tissues) and enables you to perform such applications as solid-phase cDNA library construction, Next Gene Sequencing, RNA Sequencing, Microarray, RT-PCR, cDNA microarrays, S1 nuclease analysis, affinity purification, ribonuclease protection assay, primer extension, in vitro translation experiments, RACE, northern analysis, gene cloning, and gene expression analysis, primer extension and subtractive hybridization.

- Uniformly dispersed magnetic polystyrene beads as matrix.
- The Oligo dT is covalently bound to the Enriching Beads® surface and it is possible to regenerate the beads for reuse.
- Very high, specific poly A+ binding capacity ensures maximum extraction of mRNA.
- Proprietary surface characteristics provides low nonspecific binding.
- Excellent stability slows settling in the absence of a magnetic field.
- Stability in buffer systems (pH 4 to 11) optimizes performance in most applications.
- Special surface encapsulation means no exposed iron and no interference with downstream enzymatic applications

Specifications

Beads composition	Magnetic polystyrene microspheres with surface coated oligo (dT)25
Particle size	1μm
Binding capacity	2~3 μg mRNA per mg（10~15μg mRNA per mL）
Concentration	Supplied at approximately 5mg/mL
Fill volume	2mL, 10mL, 100mL
Magnetic content	~30%
Magnetic property	Superparamagnetic
Additives	0.02% Tween20, 0.02% NaN3, PBS pH7.4
Stability	Compatible with most commonlyused detergents and biological buffer systems (pH 4 to 11). Stable in guanidine isothiocyanate, dimethyl formamide (DMF), and PCR cycling temperatures
Storage and handling	Unless otherwise stated, refrigerate (2°C to 8°C) product when not in use but do not freeze. Store upright and keep bottle tightly sealed. Mix product with gentle inversion by hand, roller or vortex mixer.

Preparation of mRNA for Downstream Applications

When using Enriching Beads® Oligo (dT) magnetic beads for northern analysis, the mRNA can be eluted directly into a loading buffer containing formamide and loaded directly onto the gel. If the mRNA is to be used in downstream enzymatic applications (cDNA synthesis, in vitro translations experiments, RT-PCR), detergents should be omitted in the final elution step. Enzymatic downstream applications are not inhibited by the presence of the beads. It is possible to construct solid-phase cDNA libraries specific for a particular cell type or tissue directly on the bead-surface. The covalently coupled oligo dT sequence is used to capture the mRNA and as a primer for the reverse transcriptase to synthesize the first strand cDNA. This results in a covalently coupled first-strand cDNA library. We recommend the beads-mRNA complex to be used immediately for RT-PCR. If storage is necessary, elute the mRNA from the beads and freeze.

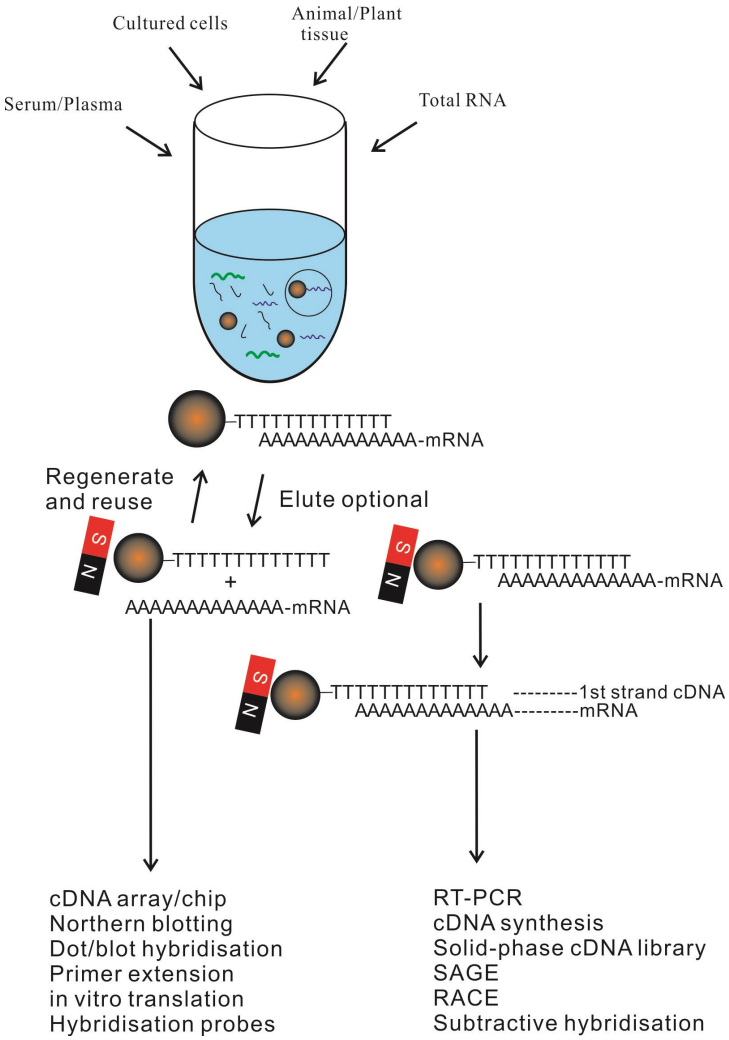

Figure 3: A flow diagram of the mRNA Isolation System and application by Enriching Beads® Oligo (dT) Magnetic Beads

Binding Capacity

2~3 μg poly(A)+ RNA can be isolated per mg of beads, depending on the tissue or cell type and the expression level of the mRNA. The total binding capacity per mL of beads is approx. 10~15 μg mRNA. Only 1-5% of total RNA content of cells is actually mRNA. Compare this to Total RNA purification methods where up to 80% of the RNA yield is ribosomal RNA. Enriching Beads® Oligo (dT) magnetic beads isolate the mRNA transcriptome when you need free of contaminating of ribosomal RNA, tRNA, miRNA, siRNA, nonPolyA RNA and preprocessed RNA. The same beads could be reused four cycles for mRNA isolations.

Troubleshooting

Problem	Possible Cause	Solution
Low mRNA yield	mRNA degraded prior to or during processing (RNase contamination from handling)	Work quickly Wear gloves throughout the procedure when handling the solutions and equipment used for RNA isolation
	RNase contamination from total RNA sample	Check total RNA sample for RNase contamination: Analyze sample by agarose gel electrophoresis
rRNA contamination	rRNA co-purified with mRNA	• Ensure total RNA samples are heated at 65°C prior to addition of magnetic beads • If the rRNA level is too high for downstream applications, purify mRNA with a second round of purification with new magnetic beads
mRNA could not be eluted	Elution condition is too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Magnetic beads aggregate	Beads were frozen or centrifuged buffer used is incompatible with magnetic beads	Handle the beads as directed in instructions
Magnetic beads are dried out	Beads were not capped correctly, or were not stored in an upright position	Resuspend the beads in the buffer they are supplied in by placing the vial on a roller or equivalent overnight (2°C to 8°C). This treatment will restore their complete functionality.
No signal observed after RT-PCR	RNA is degraded by contaminating RNases	• Use RNase-free pipette tips with aerosol barriers • Change gloves frequently • Clean pipettors with RNaseZap® solution

Ordering information

Products	Concentration	Quantity	Cat. No
Enriching Beads® MPS100/Oligo dT 1.0 μm magnetic beads with oligo dT25 coated.	5mg/mL	2mL 10mL 100mL	D30-002 D30-010 D30-100
Enriching Beads® mRNA Purification Kit			D31-002

Related products	Concentration	Quantity	Cat. No
Enriching Beads® MPS100/Streptavidin C1 1.0 μm magnetic beads with covalently coupled recombinant Streptavidin.	10mg/mL	2mL 10mL 100mL	ST01-002 ST01-010 ST01-100
Enriching Beads® MPS100/Carboxyl 1.0 μm magnetic beads with carboxylic acid groups coaded.	10mg/mL	2mL 10mL 100mL	PC11-002 PC11-010 PC11-100

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