Oligo $(dT)_{25}$ Magnetic Beads

Name	Catalog #	Concentration (mg/ml)	Volume (ml)	Amount of Beads (mg)
Oligo $(dT)_{25}$ Magnetic Beads	OTMB-100	5	2	10
	OTMB-200	5	5	25
	OTMB-300	5	100	500
	OTMB-OEM	5	≥ 1000	≥ 5000

Description

MCLAB's Oligo $(dT)_{25}$ Magnetic Beads are nano-superparamagnetic beads covalently coated with highly purified Oligo $(dT)_{25}$. Oligo $(dT)_{25}$ Magnetic Beads are polymer based affinity matrix for small-scale, rapid isolation of mRNA from crude cell lysates or tissues. The isolation occurs through the hybridization of covalently coupled oligo $(dT)_{25}$ to the poly(A) region present in most eukaryotic mRNA. The isolated mRNA can be used directly in most downstream applications in molecular biology, e.g. RT-PCR, cDNA library construction, in vitro translation experiments, and gene expression analysis.

The protocol can be performed in 15 minutes, without the need to prepare total RNA or perform any other purification steps. The Oligo $(dT)_{25}$ bound to the bead surface can be used to both capture the mRNA and act as a primer for reverse transcriptase during the first strand cDNA synthesis.

Oligo $d(T)_{25}$ Magnetic Beads are supplied as a 5 mg/ml suspension in phosphate buffered saline (PBS buffer, pH 7.4), containing 0.05% NaN_3 as preservative.

Applications

RNA Expression Analysis
RNA Detection & Isolation

Product Specifications

Bead Concentration	5 mg/ml
Bead Mean Diameter	1 µm
Binding Capacity for mRNA	~ 2 µg/mg
Storage Buffer	PBS with 0.05% NaN_3
Storage Temperature	2 to 8°C

Recommended Buffers

Lysis/Binding Buffer

100 mM Tris-HCl, pH 7.5
500 mM LiCl
10 mM EDTA, pH 8.0
1% LiDS
5 mM Dithiothreitol (DTT)

Washing Buffer A

10 mM Tris-HCl, pH 7.5
0.15 M LiCl
1 mM EDTA
0.1% LiDS

Washing Buffer B

10 mM Tris-HCl, pH 7.5
0.15 M LiCl
1 mM EDTA

Elution Buffer

10 mM Tris-HCl
10 mM Tris-HCl, pH 7.5

Protocols

The following protocols provide general guidelines and may be modified by the user for specific applications. The amount of beads should be optimized for individual application by titration.

Preparation of Oligo $(dT)_{25}$ Magnetic Beads

1. Resuspend Oligo $(dT)_{25}$ Magnetic Beads thoroughly before use.
2. Transfer desired amount of beads from stock tube to a RNasefree 1.5 ml microcentrifuge tube and place tube on a magnet.
3. After 1 minute, remove supernatant.
4. Remove tube from magnet and wash beads by resuspending in an equivalent volume of Lysis/Binding Buffer.

Isolate mRNA from Crude Lysate

1. Remove solution from washed Oligo $(dT)_{25}$ Magnetic Beads and add lysate.
2. Mix beads and lysate. Allow binding by rotating on a mixer for 10 min at room temperature. Increase annealing time if solution is viscous. During this step the mRNA anneals to the oligo dT sequence.
3. Place vial on magnet for 2 min and remove supernatant.
4. Wash beads twice at room temperature using magnet: Wash once with 1 ml Washing Buffer A, and once with 1 ml Washing Buffer B. Resuspend beads thoroughly in Washing Buffers to remove possible contaminants, and remove supernatant completely between washing steps.
5. Perform one of the following:

a. If bead-bound isolated mRNA is to be used in enzymatic downstream applications (e.g. solid-phase cDNA synthesis), wash one extra time with Washing Buffer B (0.50 ml) followed by one wash with the enzymatic buffer used in downstream application.

b. To elute mRNA from beads, remove Washing Buffer B and add 10–20 µl 10 mM Tris-HCl. Incubate at 75°C to 80°C for 2 min, then place tube on magnet and quickly transfer supernatant containing mRNA to a new RNase-free tube. The final yield may vary somewhat between tissues/cells depending on mRNA abundance.

Purify mRNA from Total RNA

In the next example, mRNA is purified from 75 µg of total RNA starting material.

1. Adjust volume of the 75 µg total RNA sample to 100 µl with distilled DEPC treated water or with 10 mM Tris-HCl pH 7.5.
2. Add 100 µl Binding Buffer. If total RNA is more dilute than 75 µg/100 µl, then simply add an equal volume of Binding Buffer to beads.
3. Heat to 65°C for 5 min to disrupt secondary structures. Immediately place on ice.
4. Add the 200 µl total RNA to 100 µl washed beads. For every 75 µg total RNA, use 1 mg beads which are washed and resuspended in 100 µl Binding Buffer.
5. Mix thoroughly and allow binding by rotating continuously on a mixer for 10 min at room temperature.
6. Place tube on magnet for 2 min and carefully remove all supernatant.
7. Remove tube from magnet and add 200 µl Washing Buffer B. Mix by pipetting several times.
8. Apply to magnet for 2 min and remove supernatant.
9. Repeat steps 7–8 once.
10. Perform one of the following:

a. If bead-bound isolated mRNA does not need to be eluted off the beads, wash one more time with the same buffer that will be used in the downstream application.

b. To elute mRNA from beads, remove Washing Buffer B and add 10–20 µl 10 mM Tris-HCl. Incubate at 75°C to 80°C for 2 min, then place tube on magnet and quickly transfer supernatant containing mRNA to a new RNase-free tube.

Precautions

1. The stable pH range of Oligo $(dT)_{25}$ Magnetic Beads is 4 to 13.
2. Do not centrifuge, dry or freeze the magnetic beads.
3. Work RNase free and wear gloves.
4. Thorough resuspension of beads/mRNA complex during washing and complete removal of washing buffer at each step will prevent carry over of LiDS and other salts to the downstream reaction. Transferring beads/mRNA complex to new tubes before the last washing step will further reduce LiDS carry over. LiDS is a strong inhibitor of enzymatic reactions.
5. For the best experimental performance, it is recommended to use a magnetic stand.
6. This product is for R&D use only, not for drug, house hold, or other uses.