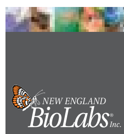
Oligo d(T)$_{25}$ Magnetic Beads



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25 mg **Lot: 0061302** **Exp: 2/16**
5 mg/ml **Store at 4°C (Do not freeze)**

Description: Oligo d(T)$_{25}$ Magnetic Beads are a 1 µm polymer based affinity matrix for the small-scale isolation of mRNA from crude cell lysates and tissue. The isolation occurs through the hybridization of covalently coupled oligo d(T)$_{25}$ to the poly(A) region present in most eukaryotic mRNA. Applications include direct mRNA isolation following lysis and second-round purification of previously isolated total RNA. The magnetic separation technology is scalable and permits elution of intact mRNA in small volumes eliminating the need for precipitation of the isolated mRNA. Beads can be reused up to three times and the researcher has the option of eluting the isolated mRNA or using the bound d(T)$_{25}$ as a primer in a first-strand cDNA reaction.

Supplied as a 5 mg/ml suspension in phosphate buffered saline (PBS Buffer) (pH 7.4), containing 0.1% Tween 20 and 0.02% sodium azide.

Support Matrix: 1 µm nonporous superparamagnetic microparticles.

Binding Capacity: 1 mg will bind 2–5 µg of poly(A)$^+$ RNA.

Recommended Buffers:

Lysis/Binding Buffer:
20 mM Tris-HCl (pH 7.5)
500 mM LiCl
0.5% LiDS
1 mM EDTA
5 mM DTT

Wash Buffer I:
20 mM Tris-HCl (pH 7.5)
500 mM LiCl
0.1% LiDS
1 mM EDTA
5 mM DTT

Wash Buffer II:
20 mM Tris-HCl (pH 7.5)
500 mM LiCl
1 mM EDTA

Low Salt Buffer:
20 mM Tris-HCl (pH 7.5)
200 mM LiCl
1 mM EDTA

Elution Buffer:
20 mM Tris-HCl (pH 7.5)
1 mM EDTA

Isolation of mRNA from Mammalian Cells:

Oligo d(T)$_{25}$ magnetic beads should be suspended by agitation at room temperature for 30 minutes prior to use.

Precautions should be taken to avoid ribonuclease contamination during the isolation procedure. Workspace should be cleaned with RNase decontaminant prior to starting the isolation.

Wear latex gloves or equivalent at all times when handling kit components.

Table 1: *Recommended Cell Isolation Scale.*

Number of Cells	Vol. of Oligo d(T)$_{25}$ Beads	Vol. of Lysis/ Binding Buffer	Wash Buffers & Low Salt Buffer	Vol. of Elution Buffer
1 x 10^5	100 µl	500 µl	500 µl	100 µl
5 x 10^5	100 µl	500 µl	500 µl	100 µl

Protocol:

Step 1

- Aliquot the appropriate volume of Oligo d(T)$_{25}$ Magnetic Beads for the scale of isolation (Table 1).

  Add 200 µl of Lysis/Binding Buffer to beads, vortex briefly and mix with agitation for 2 minutes. Beads should remain in the lysis/binding wash solution until removal immediately before adding the cell lysate.

Step 2

- **Adherent Cells**
  1. Aspirate media from cell culture plate and wash once with cold sterile 1X PBS (pH 7.4).
  2. Add the appropriate volume of Lysis/Binding Buffer (see Table 1) to cells and gently swirl by hand.
- **Suspension Cells**
  1. Pellet Cells by centrifuging at 1,000 rpm for 5 minutes at 4°C.
  2. Aspirate media and wash once with cold sterile 1X PBS (pH 7.4).
  3. Pellet again, discard PBS and add the appropriate volume of Lysis/Binding Buffer (Table 1) to cells.
  4. Agitate to suspend cells in Lysis/Binding Buffer.

Step 3

- Incubate at RT for 5 minutes with gentle agitation. If the solution is viscous, pass the lysate several times through a 21-gauge needle attached to a 1 or 2-ml syringe. A noticeable decrease in viscosity should be observed.
- Place the microcentrifuge tube containing the beads and lysis/binding wash into the magnetic rack and pull the magnetic beads to the side of the tube.

Step 4

- Remove Lysis/Binding wash and add the cell lysate to the equilibrated magnetic beads.
- Place cell lysate-and-bead suspension on the agitator and incubate at RT for 10 minutes.
- Place microcentrifuge tube into the magnetic rack and pull magentic beads to the side of tube, remove and discard supernatant.

Step 5

- Add the appropriate volume of Wash Buffer 1 (see Table 1) to the beads and mix with agitation for 1 minute.
- Place microcentrifuge tube into magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.
- Repeat Step 5, once.

Step 6

- Add the appropriate volume of Wash Buffer 2 (see Table 1) to the beads and mix with agitation for 1 minute.
- Place microcentrifuge tube into the magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.
- Repeat Step 6, once.

Step 7

- Add the appropriate volume of Low Salt Buffer (Table 1) to the beads and mix with agitation for 1 minute.
- Place microcentrifuge tube in the magnetic rack and pull magnetic beads to the side of the tube, remove and discard wash solution.

Step 8

- Add the appropriate volume of Elution Buffer (Table 1) and vortex gently to suspend beads.
- Incubate at 50°C for 2 minutes with occasional agitation to elute poly(A)$^+$ RNA. > 90% of the poly(A)$^+$ RNA bound to the beads is recovered in this step.

  Place microcentrifuge tube in the magnetic rack and pull the magnetic beads to the side of the tube. Transfer eluent to a clean, sterile RNase-free tube and store on ice for immediate quantitation or place at –80°C for long-term storage.

Quantitation of Isolated poly(A)$^+$ RNA:

Quantitation of isolated poly(A)$^+$ RNA can be determined by measurement of A_{260} nm using a Nanodrop™ spectrophotometer. The $A_{260/280}$ ratio should be 1.6 or greater.

The amount of isolated RNA will vary with the source of the RNA sample.

For RNA 1A_{260} Unit = ~ 40 µg /ml.

Notes: The eluted mRNA should be placed in the magnetic rack while removing aliquots for quantitation to avoid pipetting of beads, which will interfere with spectrophotometric analysis.

Isolated poly(A)$^+$ RNA is usually greater than 70% pure. Ribosomal RNA (rRNA) and trace amounts of genomic DNA (gDNA) are sometimes seen as impurities in isolated poly(A)$^+$ RNA. The presence of rRNA will not interfere with the analysis of results from Northern Blotting and RT-PCR experiments. Trace gDNA contamination is usually low enough to permit valid qRT-PCR quantitation of mRNA transcripts without further purification.

Reuse of Oligo d(T)$_{25}$ beads:

- Oligo d(T)$_{25}$ beads can be re-used for a second-round of purification of a poly(A)$^+$ RNA eluent without regeneration. Wash beads once with an additional 100 µl of elution buffer. Place in magnetic rack and pull beads to the side of the tube. Remove and discard wash solution. Wash beads once with 200 µl of Lysis/Binding Buffer then re-apply previously isolated eluent to beads after adjusting salt concentration to 0.5 M LiCl or 0.5 M NaCl. Repeat isolation steps.

Regeneration of Oligo d(T)$_{25}$ beads:

- Oligo d(T)$_{25}$ beads can be regenerated and

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used to isolate RNA from a different source. Add 0.1 N NaOH to the beads and incubate at room temperature with agitation for 5 minutes. Wash, equilibrate beads and store beads in sterile RNase-free 1X PBS (pH 7.4) containing 0.1% Tween 20.

Troubleshooting:

- *Viscous lysate*

  Certain cells lines and types of tissue will produce lysates that are more viscous than normal. Pass these cell or tissue lysates several times through a 21-gauge needle. If sample lysate remains viscous after additional shearing, proceed with isolation. Poly(A)$^+$ RNA can be isolated under these conditions but is more likely to be contaminated with gDNA.

- *Clumping of beads*

  Clumping of beads is usually caused by non-sheared gDNA. If lysis- bead- suspension clumps, rapidly pipette cell or tissue lysate up and down with a 1 ml pipetman to further reduce viscosity. Poly(A)$^+$ RNA can still be isolated under these conditions but is more likely to be contaminated with gDNA.

- *Genomic DNA contamination*

  Lower the ratio of cells or tissue to Lysis/Binding Buffer volume. Increase the binding incubation time if the lysate remains viscous after additional shearing. If intended downstream application requires mRNA be gDNA free, a second round of isolation should be done. See reuse of Oligo d(T)$_{25}$ Magnetic Beads.

Companion Products:

ProtoScript First-Strand cDNA Synthesis Kit
#E6500S 30 reactions
#E6500L 150 reactions

ProtoScript II RT-PCR Kit
#E6400S 30 reactions

DyNAmo™ SYBR® Green 2-Step qRT-PCR Kit
#F-430S 40 qPCR reactions
#F-430L 200 qPCR reactions

6-Tube Magnetic Separation Rack
#S1506S

12-Tube Magnetic Separation Rack
#S1509S