

Oligo (dT)₂₅ Cellulose Beads



1-800-632-7799
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www.neb.com



S1408S 007160318031

S1408S

250 mg **Lot: 0071603**
Store at 4°C **Exp: 3/18**

Description: An affinity matrix used for the isolation of mRNA containing polyadenylic (poly A) regions (1). This matrix consists of oligo (dT)₂₅ covalently coupled to a cross-linked cellulose bead. This product is guaranteed for six months when stored at 4°C in the supplied storage buffer.

Loading Buffer: **Elution Buffer:**
0.5 M NaCl 20 mM Tris-HCl, (pH 7.5)
20 mM Tris-HCl, (pH 7.5)
1.0 mM EDTA

Low Salt Buffer:
0.1 M NaCl
10 mM Tris-HCl (pH 7.5)
1.0 mM EDTA

Poly rA Binding Assay: A spectrophotometric determination of the amount of poly rA (M.W. >100,000) bound per gram of cellulose.

Poly rA Binding Capacity: > 400 A₂₆₀ units/gram

Notes:
Store at 4°C. **Do not freeze**
Supplied as a 50% (v:v) slurry

References:
1. Aviv, H. and Leder, P. (1972) *Proc. Natl. Acad.* 69, 1408.

Recommended use of Oligo(dT)₂₅ Cellulose beads:
1. Prewarm elution buffer by placing in a 70°C bath.
2. Set microcentrifuge at 2000 to 5000 x g.

- In a clean microcentrifuge tube:
Add 12.5 mg (150 µl of 50% slurry) of Oligo (dT)₂₅ Cellulose beads for each 0.5-1 mg of total RNA. Microcentrifuge for 20 seconds. Using a micropipette decant storage buffer.
- Equilibrate cellulose beads by adding 500 µl of loading buffer. Resuspend beads by gentle agitation for 2 minutes. Microcentrifuge and decant as before.
- Dissolve RNA in loading buffer and heat to 65°C for 5 minutes. Be sure that RNA is totally dissolved. Quickly cool the sample in an ice bath for 5 minutes, then add to cellulose beads.
- Allow to stand at RT for 5 minutes, agitating gently by hand or place horizontally on a rotary shaker.
- Microcentrifuge for 20 seconds, decant solution and return to original micro-centrifuge tube. Heat again to 65°C, cool and reapply to cellulose. Allow to stand with occasional agitation as before, centrifuge, then decant and save.

- Attention:**
It is recommended that no decanted eluents be discarded until entire isolation procedure is done and results evaluated.
- Wash the Oligo (dT)₂₅ cellulose beads 5X with 500 µl volumes of loading buffer, centrifuging for 20 seconds, then carefully decant each wash.
 - Using the same method wash cellulose beads once with 500 µl of low salt buffer.
 - Elute poly(A) + RNA with 2 X 250 µl washes of prewarmed elution buffer. Read A₂₆₀ units.
 - To precipitate add Sodium Acetate [3 M (pH 5.2)] to a final concentration of 0.3 M. Precipitate the RNA with 2.2 volumes of Ethanol at -20°C. Rinse the pellet in 70% Ethanol.
- RNA should be stored in 70% Ethanol at -70°C.

Regeneration of cellulose beads:
Add 2 X volume of 0.1 N NaOH to cellulose beads. Let stand with agitation for 1 hour at room temperature. Wash with H₂O until eluent is pH 7.0. Resuspend in loading buffer.

CERTIFICATE OF ANALYSIS

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