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

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

S1408S 002130716071

S1408S

250 mg **Lot: 0021307**

Store at 4°C **Exp: 7/16**

Description: An affinity matrix used for the isolation of mRNA containing polyadenylic (poly A) regions (1). This matrix consists of oligo $(dT)_{25}$ covlaently 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:

0.5 M NaCl
20 mM Tris-HCl, (pH 7.5)
1.0 mM EDTA

Elution Buffer:

20 mM Tris-HCl, (pH 7.5)

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 A_{260} 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.
3. 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.
4. Equilibrate cellulose beads by adding 500 µl of loading buffer. Resuspend beads by gentle agitation for 2 minutes. Microcentrifuge and decant as before.
5. 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.
6. Allow to stand at RT for 5 minutes, agitating gently by hand or place horizontally on a rotary shaker.
7. 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.

8. Wash the Oligo $(dT)_{25}$ cellulose beads 5X with 500 µl volumes of loading buffer, centrifuging for 20 seconds, then carefully decant each wash.
9. Using the same method wash cellulose beads once with 500 µl of low salt buffer.
10. Elute poly(A) + RNA with 2 X 250 µl washes of prewarmed elution buffer. Read A_{260} units.
11. 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 H_20 until eluent is pH 7.0. Resuspend in loading buffer.

CERTIFICATE OF ANALYSIS

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