LIFE TECHNOLOGIES

Product: Oligo(dT) Cellulose Columns

Cat. No.: 15939-010 **Storage Conditions:** -20°C

Lot No.:

Size: Five Columns (100 mg oligo(dT) cellulose per prepacked column)

DESCRIPTION:

Oligo(dT) Cellulose is an affinity matrix designed for the isolation of poly(A)+ RNA (mRNA) (1). The matrix is synthesized by a modification of the procedure of Gilham (2). Chains of deoxythymidylate up to 20 nucleotides long are covalently attached to a cellulose matrix via the terminal 5´-phosphate group.

QUALITY CONTROL DATA:

Poly(A) Binding Assay

Oligo(dT) cellulose is assayed for its ability to bind poly(A) (MW 100,000) at room temperature. Poly(A) in binding buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.3 M NaCl, 0.1% (w/v) SDS) is passed over a column of oligo(dT) cellulose. After extensive washing, the bound poly(A) is eluted with elution buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% (w/v) SDS). As quantified spectrophotometrically, the binding capacity is ≥ 60 A_{260}/gm oligo(dT) cellulose (approximately 6 A_{260}/column).

Overview of mRNA purification

Most eukaryotic mRNAs contain poly(A) tails at their 3´ termini ranging in size from 50 to 200 nucleotides (3,4) that can be used to physically select mRNA from a total RNA population by affinity chromatography (1,5). The RNA is passed over an oligo(dT) cellulose column in the presence of 0.3 to 0.5 M NaCl to promote hybridization of the poly(A) tails to the oligo(dT). After the column is washed to remove unbound nucleic acids, the mRNA is eluted by destabilizing the hybrids of oligo(dT) and poly(A). This generally is done by removing the NaCl, and occasionally by raising the temperature. This can yield an RNA preparation that contains >90% mRNA.

> RNases can be introduced adventitiously into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. Care should be taken that all buffers and glassware are sterile and free of ribonuclease activity.

Procedure

The following procedure is an example of the use of Oligo(dT) Cellulose Columns for the isolation of mRNA from a preparation of total RNA.

Isolation of mRNA from Total RNA

1. Remove the end caps from the Oligo(dT) Cellulose Column and clamp the column to a ringstand.
2. Load 1 ml of 0.1 M NaOH onto the column and let it drain completely.
3. Equilibrate the column with 4 ml of binding buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.3 M NaCl, 0.1% (w/v) SDS] (at room temperature) in 1-ml aliquots.
4. Load 1 ml of binding buffer onto the column and let about 0.5 ml pass through. The column now is ready to use. If you wish to store the column, cap the ends of the column securely and store at 4°C.
5. Add 3 ml of binding buffer to isolated total RNA pellet obtained from 1 g of tissue or wet-weight of packed cells. Dissolve by trituration using a sterile pipette.
6. Heat the RNA solution in a 70°C water bath for 5 min and chill on ice for 5 min. If the SDS in the buffer precipitates, warm it to room temperature only and swirl it until the SDS goes back into solution.
7. If there are any particulates that do not go into solution, remove them by centrifugation at approximately 1,000 × *g* for 5 min at room temperature and retain the supernatant fraction. A small, table-top clinical centrifuge is sufficient for this purpose.

Doc. Rev.: 020300

8. Dilute a 10-µl aliquot from the clarified solution with 990 µl of binding buffer. Read the A_{260} of this solution with the spectrophotometer blanked against binding buffer. Calculate the amount of RNA:

$$\text{Total RNA (mg)} = \frac{A_{260} \times 100 \times 3 \text{ ml}}{25\ A_{260}/\text{mg/ml}}$$

where: A_{260} is the absorbance of the solution measured with the spectrophotometer at 260 nm, 100 is the dilution factor, 3 ml is the total volume of the RNA solution, 25 A_{260}/mg/ml is the conversion factor relating absorbance to concentration.

If the amount of RNA exceeds 20 mg, run the Oligo(dT) Cellulose Column more than once to prevent overloading.

9. Load the dissolved RNA (≤ 20 mg) onto the column under gravity flow and wash with 4 ml of binding buffer to elute non-messenger RNA.

10. Elute the mRNA with 1.5 ml of elution buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% (w/v) SDS) and collect the eluate as one fraction in a 15-ml sterile, disposable centrifuge tube.

11. Reequilibrate the Oligo(dT) Cellulose Column with 4 ml of binding buffer.

12. Heat the RNA from step 10 in a 70°C water bath for 5 min and chill in an ice-water bath for 5 min.

13. Place the RNA solution at room temperature for 20 min, then add 90 µl of 5 M NaCl. *Immediately load the RNA onto the column and wash it with 4 ml of binding buffer.*

14. Elute the mRNA with 1.5 ml of elution buffer and collect the eluate as one fraction in a 15-ml sterile, disposable centrifuge tube.

15. Add 90 µl of 5 M NaCl and 3 ml of ethanol to the RNA. Place the tube at -20°C overnight, or until needed.

16. The Oligo(dT) Cellulose Column should now be rewashed, as in step 2, followed by washing with autoclaved distilled water until the effluent is neutral, using pH indicator strips to monitor the approximate pH. Reequilibrate the Oligo(dT) Cellulose column with 4 ml of binding buffer before reusing. This column may be used to purify mRNA from total RNA several times before it should be discarded. If you wish to store the column, cap ends of column securely and store at 4°C.

17. Centrifuge sample from step 15 at 7,000 × *g* for 20 min at 4°C and remove the supernatant fraction.

18. Carefully add 1 ml of 75% ethanol to the tube and centrifuge at 7,000 × *g* for 2 min at 4°C.

19. Remove the supernatant fraction and dry the pellet at room temperature until the ethanol has evaporated completely, approximately 30 min. This can be hastened by using a heating block at 50°C for approximately 10 min.

20. Dissolve the pellet in a minimal volume of DEPC-treated 1 mM EDTA (pH 7.5) solution (5 to 50 µl is recommended) and store at -70°C if available; if not, store at -20°C.

REFERENCES:

1. Aviv, H. and Leder, P. (1972) *Proc. Natl. Acad. Sci. USA 69*, 1408.
2. Gilham, P.T. and Robinson, W.E. (1964) *J. Amer. Chem. Soc. 86*, 4985
3. Taylor, J. M. (1979) *Ann. Rev. Biochem.* (E. E. Snell, P. D. Boyer, A. Meister and C. C. Richardson, eds.) Vol. 48, p. 681, Annual Reviews, Inc., Palo Alto, California.
4. Kedes, L. H. (1979) *Ann. Rev. Biochem.* (E. E. Snell, P. D. Boyer, A. Meister and C. C. Richardson, eds.) Vol. 48, p. 837, Annual Reviews, Inc., Palo Alto, California.
5. Jacobson, A. (1987) *Methods Enzymol.* (S. L. Berger and A. R. Kimmel, eds.) Vol. 152, p. 254, Academic Press, Orlando, Florida.