INSTRUCTIONS for Oligo(dT)-Cellulose Type 7

Oligo(dT)-Cellulose Type 7 is an affinity medium for the isolation of mRNA. It contains approximately 40 mg of oligo(dT) per gram of cellulose, and the chains of dT range from 2–25 nucleotides in length. Oligo(dT)-Cellulose Type 7 contains few fines and therefore is compatible with column chromatography.

The oligonucleotide celluloses are supplied as dry powders. We recommend storing them dry at –20 °C; however they are stable at room temperature when kept dry and free of microbial contamination.
Columns of oligo(dT)-cellulose can be used repeatedly at room temperature with little loss in the ability to bind poly(A).

Protocol for mRNA Purification Using Oligo(dT)-Cellulose Type 7

1. Suspend the appropriate amount of Oligo(dT)-Cellulose Type 7 powder in DEPC-treated sterile water. One gram of cellulose swells to 2.5–3.5 mL of gel.
2. Using autoclaved minicolumns (0.6 cm diameter), prepare two columns, each with a bed volume of 0.5 mL. This will be sufficient for isolation of polyadenylated RNA from 1–3 mg of total RNA (from 1 g of tissue or cells).
3. Wash each column with 3–5 bed volumes of 0.1 M NaOH and then with 10 bed volumes of equilibration buffer or until the pH of the eluent is 7.5.
4. Redissolve the crude RNA preparation in 0.5 mL of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA; TE buffer should be prepared using DEPC-treated water).
5. Heat the RNA at 65 °C for 5 minutes, cool quickly on ice, add 0.5 mL of 1 M NaCl, and then mix. Apply to one of the oligo(dT)-cellulose columns at room temperature.
6. Collect the eluent, heat at 65 °C for 5 minutes, cool quickly on ice and reapply it to the column.
7. Wash the column with 5–10 bed volumes of equilibration buffer, and then with 5 bed volumes of wash buffer. Elute the RNA with 3 bed volumes of TE buffer, collecting 0.2 mL fractions.
8. Identify the RNA-containing fractions as follows: spot 1–3 µL of each fraction onto a piece of plastic wrap, add 20 µL of ethidium bromide solution to each spot, place the plastic wrap on an ultraviolet light box, and then identify the RNA-containing fractions by their fluorescence. Pool these fractions.
9. Using the second oligo(dT)-cellulose column, repeat steps 5 through 8 with the pooled fractions. Pool the RNA-containing fractions from the second column.
10. Add 0.2 volumes of 2 M NaCl and 3 volumes of cold ethanol to the pooled fractions. Mix, and then chill at –20 °C for at least 2 hours.
11. Collect the RNA by centrifugation at 4 °C, remove the supernatant, dry the pellet briefly, and then redissolve it in 20 µL of TE buffer. Determine RNA concentration by spectrophotometry or ethidium bromide fluorescence.

Regeneration

Wash the columns with 0.1 M NaOH or KOH to strip off all the RNA, then follow with 5 bed volumes of 5 mM EDTA and another 5 bed volumes of DEPC-treated sterile water.

If the cellulose is going to be used immediately, reequilibrate with 5 bed volumes of equilibration buffer.

If the cellulose is not going to be used immediately, equilibrate it with sterile 0.5 M NaCl or KCl solution and store at 4 °C.
For longer storage, wash with 50% ethanol, and then dry in a vacuum. Store the dry powder at –20 °C.

Ordering Information

Product	Pack size	Product Code
Oligo(dT)-Cellulose Type 7	1 g	27-5543-02