

OLIGO (dT)-CELLULOSE COLUMNS

Cat. No. OT 125

Store at -20 C.

PRODUCT DESCRIPTION

The Oligo (dT) - Cellulose Columns (cat. no. OT 125) are designed for fast and reliable isolation of the poly A⁺ fraction from total RNA. The columns contain oligo (dT) chains up to 30 nucleotides in length, covalently linked via the terminal 5' phosphate to the cellulose matrix. This matrix is layered with an anti-clogging resin to maintain a high flow rate. Each column has the capacity to isolate up to 50 µg of poly A⁺ RNA from 1.0 - 1.5 mg of total RNA. Each kit contains 5 columns, 500 µl of PolyAcryl Carrier (cat. no. PC 152) for mRNA precipitation, and tubing to adapt a syringe or micropipette to fit the column opening. Each column is reusable up to 10 times, making this method the most economical procedure available for poly A⁺ selection. Poly A⁺ selection can be completed in approximately 20 minutes. Total RNA can be obtained using the TRI Reagent[®] (cat. no. TR 118) method or other procedures.

PICTURE OF ISOLATED mRNA



Non-radioactive hybridization of GAPDH mRNA
isolated from kidney, heart, brain and small intestine
(lanes 1-4 respectively; 2 µg per lane).

PROTOCOL

The isolation of poly A⁺ RNA is based on the most recent modifications (1, 2) of the standard Aviv and Leder protocol (3).

REAGENTS

Binding buffer: 0.5 M LiCl, 50 mM sodium citrate, 0.1% SDS. (no pH adjustment required)

Elution buffer: 1 mM sodium citrate, 0.1% SDS. (no pH adjustment required)

Other reagents: 1% SDS, 75% ethanol, 1 M tris (free base), 5 M LiCl and isopropanol.

All aqueous reagents and glassware should be made RNase-free by DEPC-treatment (1, 2). Prepare 75% ethanol using DEPC-treated water.

1. COLUMN PREPARATION.

Immediately before use, wash columns with 0.5 ml binding buffer.

2. ISOLATION OF POLY A⁺RNA (BASIC PROTOCOL).

Following precipitation with isopropanol, dissolve the pellet of total RNA (TRI Reagent RNA isolation protocol, step 3) in the binding buffer to a concentration of 1-4 mg RNA/ml and incubate the solution at 70 C for 5 minutes. The incubation at 70 C denatures the secondary structure of RNA and increases retention of the poly A⁺ RNA on the column. Apply the RNA solution on the column, collect the eluate and apply it again on the column. Wash the column two times with 1 ml of the binding buffer. Elute poly A⁺ RNA with 0.9 ml of the elution buffer into a 2 ml microcentrifuge tube.

3. PRECIPITATION.

Take a 20-50 μ l aliquot of the eluate for spectrophotometric measurement of the RNA concentration. Next, supplement the eluate with LiCl to a final concentration of 0.5 M and with 2 - 8 μ l of PolyAcryl Carrier, included. Mix the solution briefly and precipitate RNA by adding one volume of isopropanol (or 2.5 volumes of ethanol) and incubating the sample for 5 minutes at room temperature. Centrifuge the RNA-carrier precipitate at 12 000 g for 5 minutes at 4 C. Dissolve the resulting pellet in FORMAZol[®] (cat. no. FO 121) or in DEPC-treated water.

4. YIELD.

A single pass of total RNA through the MRC oligo (dT)-cellulose column results in a retention of about 85% of poly A⁺ RNA. A second pass of total RNA through the column increases total retention of poly A⁺ RNA to over 90%. Using oligo (dT) cellulose, 2% - 5% of total RNA applied is retained as a poly A⁺ enriched fraction. Approximately half of this fraction is poly A⁺ RNA with the remainder being ribosomal RNA (1, 2). For a typical northern blot apply 1-3 μ g of the poly A⁺ fraction per lane. Using a hybridization solution containing dextran sulfate such as High Efficiency Hybridization System (cat. nos. HS 114 and HS 114F) and a probe labeled to a specific activity of 10⁹ dpm/ μ g DNA, beta-actin mRNA can be detected after 0.5 h autoradiography.

5. COLUMN REGENERATION.

Following elution of the poly A⁺ fraction, wash columns with 1.5 ml of 1% SDS, followed by 1 ml of 75% ethanol and store columns wetted with ethanol at -20 C. In case of contamination, the columns can be washed with 1.5 ml of 0.1 M NaOH followed by wash with water until the eluate reaches a pH below 7.5. Columns can be stored for at least 2 years at -20 C. Each column can be reused at least ten times.

6. ISOLATION OF POLY A⁺ RNA FROM SMALL AMOUNTS OF (<0.5mg) OF TOTAL RNA (Replace Step B with Step F.).

Mix one volume of the aqueous phase (TRI Reagent, RNA isolation protocol, step 3) with 0.1 volume of 1 M Tris (free base) and 0.8 volume of isopropanol. Store the resulting suspension for 5 minutes at room temperature and apply it on the MRC oligo (dT)cellulose column. After all the solution has passed through the column, wash the column with 0.5 ml 75% ethanol followed by 1.0 ml of the binding buffer. Collect the binding buffer, heat it at 70 C for 5 minutes and pass it through the column a second time. Wash the column with another 1.0 ml of the binding buffer and elute the poly A⁺ fraction with 0.9 ml of elution buffer.

The entire process of oligo (dT)-cellulose chromatography can be completed in 15-20 minutes. Usually, no external pressure is necessary to pass the solution through the column. In case of problems with flow, apply gentle pressure on the column using a 1 ml micropipette. If your micropipette end does not fit the column, use the tubing provided to adapt any syringe or micropipette to fit the column opening.

REFERENCES

1. Sambrook J, Fritsch E F, Maniatis T. 1989. Selection of poly A+ RNA. In: Molecular Cloning, vol.1, 7.26-7.29.
2. Kingston R E. 1991. Preparation of poly A+ RNA. In: Current Protocols in Molecular Biology (Ausubel F, et al., eds), vol. 1, 4.5.1-4.5.2.
3. Aviv H, Leder P. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc Natl Acad Sci USA 69, 1408-1412.