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

Description

MCLAB's Oligo $(dT)_{25}$ Agarose Beads are intended for the enrichment of poly(A) mRNA from total RNA preparations. 1 ml of Oligo $(dT)_{25}$ Agarose binds approximately 1 mg of poly(A) RNA. Oligo $d(T)_{25}$ Agarose Beads contain few fines and therefore are compatible with column chromatography. Supplied as a 50% suspension in 20% ethanol.

Applications

RNA Expression Analysis
RNA Detection & Isolation

Product Specifications

Matrix	Highly cross-linked 6% beaded agarose
Bead Geometry & Size	Spherical 50 to 150 µm
Bead Mean Diameter	90 µm
Binding Capacity for mRNA	~ 1 mg/ml
Storage Buffer	20% ethanol
Storage Temperature	2 to 8°C

Recommended Buffers

Binding Buffer:	0.5 M NaCl, 0.01 M Tris-HCl pH 7.5, 0.5% SDS, 0.1 mM EDTA
Wash Buffer:	0.2 M NaCl, 0.01 M Tris-HCl pH 7.5, 1 mM EDTA
Elution Buffer:	0.01 M Tris-HCl, pH 7.5, 1 mM EDTA
TE Buffer:	10 mM Tris-HCl, pH 7.5, 1 mM EDTA

Protocols

The following protocols provide general guidelines and may be modified by the user for specific applications. The amount of beads should be optimized for individual application by titration.

mRNA Purification Using Oligo $(dT)_{25}$ Agarose

1. 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).
2. Wash each column with 3–5 bed volumes of 0.1 M NaOH and then with 10 bed volumes of Wash Buffer or until the pH of the eluent is 7.5.
3. Redissolve the crude RNA preparation in 0.5 ml of TE Buffer.
4. 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 $(dT)_{25}$ Agarose columns at room temperature.
5. Collect the eluent, heat at 65°C for 5 minutes, cool quickly on ice and reapply it to the column.
6. Wash the column with 5–10 bed volumes of Wash Buffer. Elute the RNA with 3 bed volumes of TE Buffer, collecting 0.2 mL fractions.
7. 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.
8. Using the second Oligo $(dT)_{25}$ Agarose column, repeat steps 4 through 7 with the pooled fractions. Pool the RNA-containing fractions from the second column.
9. 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.
10. 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 column is going to be used immediately, reequilibrate with 5 bed volumes of Wash Buffer.

If the column 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 store the column at 4°C in 20% ethanol.

Precautions

1. The stable pH range of Oligo $(dT)_{25}$ Agarose Beads is 4 to 13.
2. Do not freeze the agarose beads.
3. Work RNase free and wear gloves.
4. This product is for R&D use only, not for drug, house hold, or other uses.