Invitrogen
life technologies

cDNA Size Fractionation Columns

Cat. No. 18092-015 **Size: 3 Columns**
Store at 4°C.

Description:
cDNA Size Fractionation Columns are 1 ml, prepacked, disposable columns for removal of small DNA (< 500 bp) and size fractionation of cDNA (> 500 bp). Each column contains 1 ml of Sephacryl® S-500 HR in a 2 ml column.

Recommended Protocol:
Selection of appropriate fractions from the column is critical to the success of a cDNA library. For more details please refer to the manual in the SuperScript™ Plasmid (Cat. No. 18248-013) or Choice (Cat. No. 18090-019) cDNA systems.

The columns are packed in 20% ethanol which must be completely removed prior to applying the sample. The washing steps will take approximately 1 h. The presence of a top frit prevents disruption of the resin bed during the washing and loading steps as well as ensuring that the resin does not dry out.

1. Place column in a support and remove the top cap first, and then the bottom cap. Allow the excess liquid to drain.

2. Pipet 0.8 ml TEN buffer [10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 25 mM NaCl] onto the upper frit, and let it drain completely. Repeat this step three more times.

3. Label 20 sterile microcentrifuge tubes from 1 to 20.

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4. Add the entire ^{32}P-labeled cDNA sample in 100 µl of TEN buffer and let it drain into the bed. Collect the effluent into tube 1.

5. Add 100 µl of TEN buffer to the column, and collect the effluent into tube 2. **Note**: Let the column drain completely before the addition of each new 100-µl aliquot.

6. Beginning with the next 100-µl aliquot of TEN buffer, collect single-drop (≈35 µl) fractions into individual tubes.

7. Continue adding 100-µl aliquots of TEN buffer until you have collected a total of 18 drops.

8. Measure the volume in each tube (using a fresh pipette tip for each fraction) and obtain Cerenkov counts for each sample. Do not add scintillation fluid to the tubes.

9. cDNA for cloning should appear after passage of 400 to 450 µl of buffer. Do not use fractions after passage of 550 µl of buffer, as these will contain a higher percentage of small cDNA or adapters.

10. cDNA may be used directly in ligation reactions or fractions may be pooled and precipitated.

Quality Control:
This product has passed the following quality control assays: flow rate, drop volume and separation of bromophenol blue and xylene cyanol FF dyes.