Mini Column Fractionation Kit

Description

Mini Column Fractionation Kit 69995-3

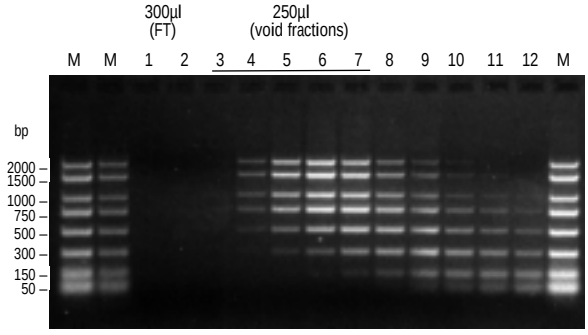
The Mini Column Fractionation Kit is designed for rapid, effective size fractionation of DNA and removal of small molecules from DNA solutions by gel filtration. The Column Buffer is compatible with direct alcohol precipitation of nucleic acids following the optional addition of glycogen as a carrier. When used as directed belowDNA fragments greater than 300bp in size are collected in the void fractions. A variety of fractionation schemes can be used by adjusting the fraction sizes and analyzing samples by gel electrophoresis. A sample fractionation profile of specific DNA fragments is shown below.

Components

- 5ml Gel Filtration Resin
- 5 Mini Columns
- 5ml 10X Column Buffer (10X=3M Na acetate)
- 250µl 10mg/ml Glycogen
- 1ml TE Buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA)

Protocol

1. Gently mix the bottle of Gel Filtration Resin by inversion until completely suspended. Transfer slurry (1ml settled bed volume, approximately 2.0ml of the slurry) to a mini column. You may need to push gently on the top of the column (using a gloved finger or Parafilm) to start the column flowing. Allow the resin to pack under gravity flow.
2. When the storage buffer drops to the top of the column bed, equilibrate the column with 5 × 1ml of 1X Column Buffer.
3. Allow the Column Buffer to drain to the top of the column bed. Carefully pipet the sample onto the column. The sample can be in any buffer such as TE. Up to 100µl can be loaded on a 1ml column.
4. After the sample has settled into the resin, gently add 200µl 1X Column Buffer without disturbing the gel bed surface. Allow the buffer to flow through.
5. Add 250µl 1X Column Buffer and collect the eluate. This is the void fraction of a 1ml column and represents the largest cDNA molecules (see figure below).
6. Add 1µl 10mg/ml Glycogen and 150µl isopropanol to the eluate. Vortex to mix and incubate at room temperature for 5 min.
7. Centrifuge at 12,000 × g for 10 min, remove the supernatant and rinse the pellet successively with 0.5ml 70% ethanol and 0.5ml 100% ethanol. Allow the pellet to dry and resuspend in 20µl TE. Store at -20°C.



DNA Marker Size Fractionation Profile

Novagen's PCR Markers (50-200bp, 100µl sample) were run on a 1ml Gel Filtration Column in Column Buffer. After collecting an initial 250µl fraction (Lane 1), subsequent 50µl fractions were collected and samples of each analyzed by agarose gel electrophoresis and ethidium bromide staining. M = markers (loaded), 1-12 = column fractions