PRINCETON SEPARATIONS

CENTRI-SEP™ 8 STRIPS

Dye Terminator Cleanup in 8-Well Format

For Research Use Only

PRINCIPLE

CENTRI-SEP™ 8 is used for the fast and efficient purification of large molecules (proteins, nucleic acids, complex carbohydrates, etc.) from small molecules (nucleotides, buffer salts, etc.). The column design is based on the description by Sambrook, et al.(1) of gel filtration for the purification of DNA from nick translation reactions. Each unit consists of a special fritted 8-column microfuge tube set containing hydrated gel designed for this purpose.

The gel will provide excellent recovery of DNA fragments **>16 base pairs or 25-mer** while removing >98% of salts, NTP's and other low-molecular-weight compounds.

The Centri-Sep™ 8 is a strip of 8-columns with hydrated, preservative-free separation matrix that is ready for sample application following a 2 minute spin. Centri-Sep™ 8 comes packaged with 12 strips sealed with foil in a single block. The block must be separated into single strips before the sealing foil is removed. Centri-Sep™ 8 strips must be brought to room temperature for at least two hours before use.

CENTRI-SEP™ 8 has been designed specifically for the following uses:

- Purification of fluorescent reaction mixtures, as in DNA sequencing with the **ABI models 373A, 377A, 310, 3100, 3700 and 3730.**
- Removal of free and labeled dNTP's from DNA/RNA as in:
  - nick translation
  - end-labeling reactions
  - polymerization reactions
- Desalting, removal of traces of phenol, or exchange of buffer salts, as in multiple restriction digestions
- Purification/desalting of proteins

These columns are far superior - in **ease of use, speed, and non-toxicity** - to such common techniques as phenol/chloroform extractions and ethanol precipitations.

Benefits include:

- **RAPID AND EFFICIENT SEPARATIONS**
- **OPTIMIZED FOR CENTRIFUGE USAGE**
- **HYDRATED AND PRESERVATIVE FREE**
- **CONVENIENT 20 µL SAMPLE SIZE**

CENTRIFUGE NOTES

Maximum yield and efficiency are obtained with the horizontal or swinging-bucket rotors. However, fixed-angle-rotor microcentrifuges provide acceptable performance and save time.

On a variable speed microcentrifuge, DO NOT use the pulse button, which overrides the speed setting and takes the rotor to maximum g-force. If you are not sure of the g-force generated by your centrifuge at specific speeds, calculate the correct speed by using the following formula:

$$rpm = \sqrt{\frac{RCF}{(1.119\ x\ 10^{-5})(r)}}$$

Where *rpm* = revolutions per minute
RCF = Relative Centrifugal Force, and
r = radius (cm) measured from center of spindle to bottom of rotor bucket.

Example:

For RCF = 750 and r = 7.5 cm

$$rpm = \sqrt{\frac{750}{(1.119\ x\ 10^{-5})(7.5)}} = \textbf{2990 rpm}$$

QUALITY CONTROL

Every batch of CENTRI-SEP™ 8 is tested for separation efficiency and fill accuracy.

MATERIALS PROVIDED

- CENTRI-SEP™ 8 Hydrated Strip (12 Strips)

ADDITIONAL MATERIALS RECOMMENDED

- Deep 96-Well (500-800 µl) plate wash plates (**sold by Princeton Separations under catalog number CS-962**)
- 96-Well PCR Plate
- 8-Well PCR strip (200 µL)

Note: These are not provided.

COMMON PROBLEMS & SOLUTIONS

•A failure to remove excess interstitial fluid before sample addition to the columns.
Common Solution: Observe if any columns have released less fluid than the others during the first spin. Simply spinning them again briefly will usually remove the excess fluid.
•Touching the inside wall of the column during sample application.
Common Solution: Load the sample directly into the center of the gel bed and do not contact the sample or the pipette tip to the walls of the column.

REFERENCE

1 Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1989.

ORDERING INFORMATION

CATALOG NO.	SIZE
CS-912	12 x 8 well strips

79-100613E

PRINCETON SEPARATIONS

CENTRI•SEP™ PROTOCOL

CENTRI-SEP™ 8 strips are recommended by Applied Biosystems, Inc. for effective and reliable removal of excess DyeDeoxy™ terminators from completed DNA sequencing reactions. The procedure below is intended to be used in conjunction with the Tag DyeDeoxy™ and ABI Prism™ terminator cycle sequencing kits, including those with AmpliTaq®, FS, used on the ABI models 373A, 377A, 310, 3100, 3700 and 3730.

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PROTOCOL for 20 µL SEQUENCING REACTION VOLUMES

1.0 Separate the desired number of strips for use by cutting the foil between strips with a blade (Fig 1). Avoid bending the block of strips since this may weaken the foil seal and cause columns to dry out.

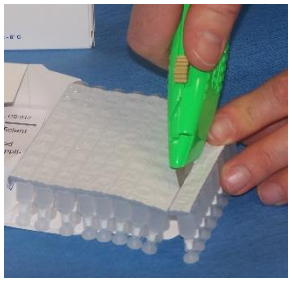

Fig 1

2.0 Open the column outlets on each strip by cutting through the bottom plastic seals with scissors. (Fig 2)

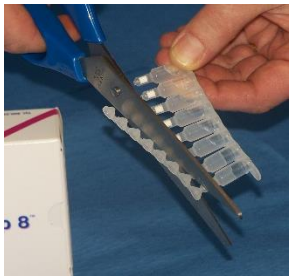

Fig 2

3.0 Remove the foil from the top of the strip. (Fig 3)

The 8-column strips perform best when centrifuging is oriented vertical to the axis of rotation.

Fig 3

4.0 Spin the strip (or strips) for 2 minutes at 750 x g in a swinging bucket centrifuge to remove interstitial fluid in either of two ways:

4.1 The 8-Column strips should be positioned in **A6→H6, A7→H7** direction as close to the center as possible, when placed in microplate carriers found in "H" shaped rotors. (Fig 4)

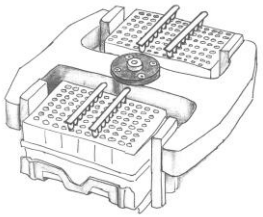

Fig 4

4.2 The 8-Column strips should be positioned in **D3→D10, E3→E10** direction as close to the center as possible, when placed in microplate carriers in rotors similar to snowflake rotors. (Fig 5)

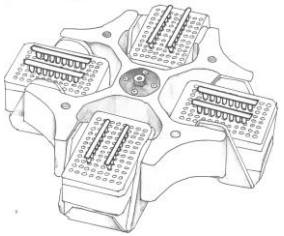

Fig 5

5.0 After centrifugation, the gel should be packed into the column and is ready to accept the sample (Fig 6)

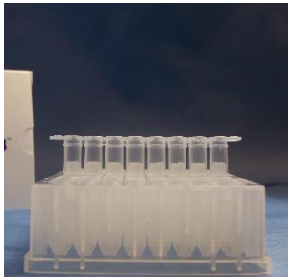

Fig 6

6.0 Add up to 20 µL of sample directly to the center of each well of Centri-Sep™ 8 column using either a mult-channel or single channel pipettor. (Fig 7)

Fig 7

7.0 Place the loaded Centri-Sep™ 8 into an 8-well PCR strip or 96-well PCR plate and spin for 2 minutes at 750 x g to collect the sample. Deep 96-well well plates (500-800 µL volume) are convenient for holding Centri-Sep™ 8 columns for both the initial spin and for the sample collection spin.

8.0 Discard the Centri-Sep™ 8 columns.

Princeton Separations, Inc. P.O. Box 296, Adelphia, NJ 07710 Phone (800) 223-0902, www.prinsep.com