

CENTRI-SEP COLUMNS

For Research Use Only

PRINCIPLE

CENTRI-SEP Columns are 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 microfuge tube, dry gel, a wash tube and a sample collection tube - all designed for this purpose.

The gel will provide excellent recovery of DNA fragments ≥ 16 base pairs or oligonucleotides ≥ 25 -mer while removing $>98\%$ of salts, NTP's and other low-molecular-weight compounds from the sample. The column gel is hydrated with reagent grade water or a suitable buffer and spun in a microcentrifuge or swinging-bucket centrifuge to remove the interstitial fluid. The sample is then applied and the column is spun again, processing the sample. The sample is thus purified by removing low molecular weight components and exchanged into the buffer of choice.

CENTRI-SEP Columns have been designed specifically for the following uses:

- Purification of fluorescent reaction mixtures, as in DNA sequencing with the ABI 373A and 377A
- 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

Use of these columns is 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
- BUFFER NOT PRESELECTED
- COLUMNS STABLE AT ROOM TEMPERATURE
- CONVENIENT 20-100 μL SAMPLE SIZE

PROCEDURAL 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:

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

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
r = 7.5 cm:

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

QUALITY CONTROL

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

MATERIALS PROVIDED

- CENTRI-SEP Columns containing dry gel
- Wash Tubes (2 mL)
- Sample Collection Tubes (1.5 mL)

ADDITIONAL MATERIALS RECOMMENDED

- Microcentrifuge (Eppendorf 5415C, Variable Speed or equivalent)
- Variable pipets (Pipetman 100 µl)
- Pipet Tips
- Pipet Bulb, Dispo, 2ml Latex
- Microtube Rack
- Vortex Mixer

COMMON PROBLEMS OFTEN RESULTING IN INEFFECTIVE SEPARATION

1) A failure to remove excess interstitial fluid after hydration of the columns.

Solution: Note 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.

2) Touching the side of the column during sample application.

Solution: Load the sample directly into the center of the gel bed and do not touch the sample 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.

<u>CATALOG NO.</u>	<u>SIZE</u>
CS-900	32 pack
CS-901	100 pack

CENTRI-SEP Protocol

Removal of Dye Terminators Prior to Sequencing

CENTRI-SEP Columns 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 Taq DyeDeoxy™ and ABI Prism™ terminator cycle sequencing kits, including those with AmpliTaq®, FS, used on the ABI 373A or 377A Sequencer.

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1.0 Column Hydration

- 1.1 Gently tap the column to insure that the dry gel has settled in the bottom of the spin column.
- 1.2 Remove the top column cap and reconstitute the column by adding 0.80 mL of reagent grade water or buffer. Leave the column end stopper in place so the column can stand up by itself. Replace the column cap and hydrate the gel by shaking and inverting the column or vortexing briefly. It is important to hydrate all of the dry gel.
- 1.3 Allow at least 30 minutes of room temperature hydration time before using the columns. Reconstituted columns may be stored refrigerated at 4°C for several days. Longer storage can be accomplished in 10 mM sodium azide (NaN₃). Allow refrigerated columns to warm to room temperature before continuing this procedure.

2.0 Removal Of Interstitial Fluid

- 2.1 Remove air bubbles from the column gel by inverting the column and sharply tapping the column, allowing the gel to slurry to the opposite end of the column. Stand the column up and allow the gel to settle while in a microtube rack.
- 2.2 After the gel has settled and is free of bubbles, first remove the top column cap, and then remove the column end stopper from the bottom.
- 2.3 Allow excess column fluid to drain (gravity) into a WASH TUBE (2 mL). If the fluid does not begin to flow immediately through the end of the column, use a 2 mL latex pipet bulb to apply gentle air pressure to the top of the column to force the fluid to start through the column filter. The column will stop draining on its own. Approximately 200–250 µL will drain from the column. Discard this fluid.
- 2.4 Spin the column and wash tube in a variable speed centrifuge at 750 × g for 2 minutes to remove interstitial fluid. For example, using the Eppendorf microcentrifuge, Model 5415C at 750 × g (7.3 cm rotor), the correct speed is 3,000 rpm. If you use a microcentrifuge, it is important to keep track of the position of the column using the orientation mark molded into the column.
- 2.5 Approximately 300 µL of fluid will be removed. If there is a drop at the end of the column, blot it dry. Discard the wash tube and the interstitial fluid. Do not allow the gel material to dry excessively. Process the sample within the next few minutes.

3.0 Sample Processing

- 3.1 Hold the column up to the light. Transfer 20 μL of completed DyeDeoxy™ terminator reaction mixture to the top of the gel. Carefully dispense the sample DIRECTLY ONTO THE CENTER OF THE GEL BED at the top of the column, without disturbing the gel surface (See Figure 1). DO NOT contact the sides of the column with the reaction mixture or the sample pipet tip, since this can reduce the efficiency of purification and possibly ruin the analysis due to excess dyes.
- 3.2 Place the column into the SAMPLE COLLECTION TUBE (1.5 mL) and place both into the rotor. Maintain proper column orientation. The highest point of the gel media in the column should always point toward the outside of the rotor (See Figure 1). Spin the column and collection tube at $750 \times g$ for 2 minutes. The purified sample will collect in the bottom of the Sample Collection Tube. Discard the spin column and proceed with the ABI sample preparation procedure.
- 3.3 Dry the sample in a vacuum centrifuge. Do not apply heat.

*DyeDeoxy™, Prism™, and AmpliTaq® are trademarks of Applied Biosystems, Inc.

Figure 1

