

CentriSep™ Columns Protocol:

Removal of Dye Terminators prior to sequencing:

CentriSep 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.

CentriSep is designed for research use only.

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 up 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 column and wash tube in a variable speed centrifuge at 750 x g for 2 minutes to remove interstitial fluid. For 7.5 cm rotors (Standard table top centrifuges) 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 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.
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 dye.
- 3.2 Place the column into the **SAMPLE COLLECTION TUBE** (1.5 ml) and place both into the rotor. **Maintain proper column orientation (molded mark)**. The highest point of the gel media in the column should always point toward the outside of the rotor. Spin the column and collection tube at **750 x 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.

