Alternate Procedure to Eliminate Unincorporated dye terminators with Spin Column and 96-Well Spin Plate clean-up products

Overview

This section describes an alternate procedure for use with spin columns and spin plates for purifying extension products.

If unincorporated dye terminators are not completely removed by spin column or 96-well plate column alone, an additional SDS heat treatment can effectively eliminate unincorporated dye terminators.

Recommended Spin Columns

We recommend Centri-Sep™ Spin columns

(Applied Biosystems, P/N 401763 for 32 columns and P/N 401762 for 100 columns).

Optimizing Spin Column Purification

IMPORTANT For use with the BigDye Terminators v3.0, hydrate each column for **2** hours.

Tips for optimizing spin column purification:

- Use one column for each sample.
- Do not process more columns than you can handle conveniently at one time.
- Load the sample in the center of the column bed. Make sure that the sample does not touch the sides of the column and that the pipet tip does not touch the gel surface.

  If samples are not properly loaded, peaks from unincorporated dye terminators can result.
- Spin the column at 325-730 x g for best results. Use the following formula to calculate the best speed for your centrifuge:

  $g = 11.18 \times r \times (\text{rpm}/1000)^2$

  where:

  g = relative centrifugal force

  rpm = revolutions per minute

  r = radius of the rotor in cm
- Do not spin for more than 2 minutes.
- Perform the entire procedure without interruption to ensure optimal results. Do not allow the column to dry out.

Performing Spin Column Purification

To prepare extension products for spin column and plate purification:

Step	Action
1	Prepare 2.2% SDS (sodium dodecyl sulfate) in deionized water. This SDS solution is stable at room temperature. **CAUTION CHEMICAL HAZARD. SDS** may cause irritation to eyes, respiratory system and skin. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.
2	Add an appropriate amount of 2.2% SDS solution to your sample to bring the final SDS concentration to 0.2%. Example: Add 2 uL of 2.2% SDS to each 20 uL completed cycle sequencing reaction. Seal the tubes with caps and mix thoroughly.

3	Heat to 98C for 5 minutes, and allow tubes to cool to ambient temperature prior to proceeding to the next step. A convenient method to perform this heating is as follows: Place the tubes in a thermal cycler with the parameters: 98C for 5 minutes 25C for 10 minutes
4	Spin down the contents briefly. Prepare spin columns or spin plate. Refer to the instructions in the BigDye Terminator v3.0 protocol manual. Proceed to perform purification.

Performing Spin Column Purification

To perform purification with spin columns:

Step	Action
1	Remove the extension reaction/SDS mixture from its tube and load carefully onto the center of the gel material. **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from under the mineral oil.
2	Spin the column in a microcentrifuge at 730 x *g* for 2 minutes. **Note** If using a centrifuge with a fixed-angle rotor, place the column in the same orientation as it was in for the first spin. This is important because the surface of the gel will be at an angle in the column after the first spin.
3	Discard the column. The sample is in the sample collection tube.
4	Dry the sample in a vacuum centrifuge for 10-15 minutes without heat, or until dry. Do not over-dry.

Performing 96-Well Spin Plate Purification

To improve the purification, follow “To prepare extension products for spin column and plate purification” protocol as above then refer to the manufacturer’s instructions for the purification procedures.