

# CentriSep™ 96 plates Protocol:

## Removal of Dye Terminators prior to sequencing:

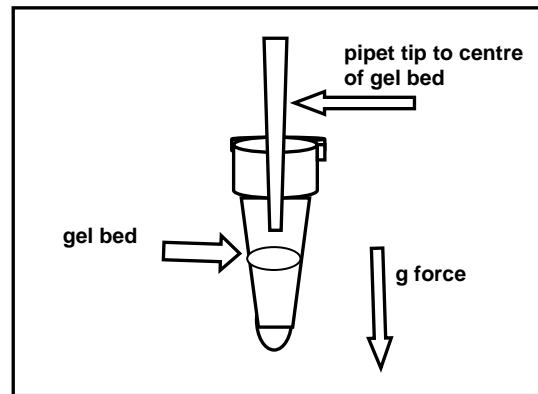
CentriSep columns are recommended by Applied Biosystems, Inc. for effective and reliable removal of excess DyeDeoxy™ terminators from completed DNA sequencing reactions. The procedure below is intended to be used in conjunction with the Taq DyeDeoxy™ and ABI Prism™ terminator cycle sequencing kits, including those with AmpliTaq®, FS, used on the ABI 373A or 377A sequencer.

### CentriSep is designed for research use only.

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- 1 **Gel is already hydrated!**
- 2 Allow plates at least 30 minutes to come to room temperature.  
  
**Allow refrigerated columns to warm up to room temperature before continuing this procedure!**
- 3 Remove the adhesive foil sealing film from the bottom and then the top of the CentriSep 96 well plates.
- 4 Stack the CentriSep 96 plate on top of a 96-well wash plate and centrifuge at 1500xg for 2 minutes. Use an external timer and start timing when rotor has reached the set speed.  
  
Discard the liquid by shaking the wash plate dry.  
  
The gel matrix in the wells should appear opaque at this point!
- 5 Transfer samples (20µL or less) to the individual wells in the CentriSep 96 well plate taking care to place the samples in the centres of the gel beds.
- 6 Stack the CentriSep 96 plate on top of a 96-well collection plate and centrifuge at 1500xg for 2 minutes (use an external timer and start timing when rotor has reached the set speed!).

- 5 Transfer samples (20µL or less) to the individual wells in the CentriSep 96 well plate taking care to place the samples in the centres of the gel beds.
- 6 Stack the CentriSep 96 plate on top of a 96-well collection plate and centrifuge at 1500xg for 2 minutes (use an external timer and start timing when rotor has reached the set speed!).
- 7 Remove the 96 well collection plate containing the cleaned samples and dry in Speed-Vac equipped with the appropriate rotor. Alternatively, the plate can be sealed with the sealing film for storage.



## Additional Notes

### Centrifugation

Most centrifuges, either bench or floor models, which accept micro-plate rotors may be used with the CentriSep 96 protocol. However, the rotor must accept a plate stack approximately 5.1cm in height (combined height of CentriSep 96 plate and wash plate) as the carrier swings 90° from its horizontal position to the vertical position.

## Additional Notes

### Timing

It is very important to control both the centrifuge speed and the duration of the run. Centrifuges vary by manufacturer with respect to when the internal timers start. Some models begin counting down as soon as the centrifuge run is started so that the ramp up to desired speed is included in the run time. If the ramp up is slow, the total time at the selected rpm is reduced, thus reducing the total g-force on the plates. We recommend the following procedure:

1. Use an external timer to monitor the centrifuge run
2. Start the timer after the rotor has reached the set speed.
3. Set the brake on maximum
4. Switch off the centrifuge after 2 minutes at 1500xg

As a visual check on centrifugation effectiveness, the matrix in the wells should appear opaque and slightly pulled away from the wall after the initial spin prior to sample application. If the matrix appears translucent or shiny, the initial centrifugation conditions are incorrect. Re-spin the plates at 1500xg for 2 minutes.

### Cushions

Cushions supplied with the centrifuge should be used under the wash plates at all times.

## Storage and Stability

### CentriSep 96 well plates must be allowed to equilibrate at room temperature before use!

We recommend that the plates for the day be removed from the refrigerator about the same time the sequencing reactions are initiated. This will allow sufficient time for the plates to warm.

The CentriSep 96 well plates should be stored at 4°C and are stable until the indicated expiration date.

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