Technical Tip

T-2

Use of CENTRI·SEP 96 filter plates for cleanup of Dye Terminator Sequencing Reactions

Introduction

CENTRI•SEP 96 *multi-well plates are an excellent solution to the difficulties encountered in removal of excess dye labeled terminators from dye terminator reactions. The hydrated matrix in these plates has been optimized for use with Big Dye reactions. When properly used,* ***CENTRI•SEP 96*** *plates will yield sequencing reactions equivalent in cleanliness to single* ***CENTRI•SEP*** *spin columns. Gel images obtained using reactions prepared with* ***CENTRI•SEP 96*** *plates will be free of interfering smears, blobs, and gel haze caused by the presence of excess dyes. Sequences may be read from the first base and longer read lengths may be obtained due to reduced background. These improved reads result in a lower cost per base than less effective precipitation methods.*

Achieving results such as described above requires optimization and standardization of centrifugation and sample loading methods. Some suggestions for these steps are given below.

Important Considerations

Allow plates to come to room temperature

Use an external timer to monitor the centrifuge run.

Start the timer after the rotor has reached the set speed.

Set the brake on maximum

Switch off the centrifuge after 2 minutes at 1500 x g.

Allow sample to "touch-off" the pipet tips rather than "blowing out" sample

Operating Temperature

CENTRI•SEP 96 plates *must* be allowed to come to room temperature before use. Equilibration requires about two hours. It is convenient to remove the plates from the refrigerator and the foil pouch when the cycle sequencing reactions are started.

Centrifugation

Most centrifuges, either bench or floor models, that accept microplate rotors may be used with the **CENTRI•SEP 96** protocol. However, the rotor must accept a plate stack approximately 4.5 cm in height (combined height of CENTRI·SEP 96 plate and wash plate) as the carrier swings 90° from its horizontal position to the vertical position.

PRINCETON SEPARATIONS

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Timing

It is very important to control both the centrifuge speed and the duration of the run. Centrifuges vary by manufacturer in exactly when the internal timers start. Some models begin counting down as soon as the centrifuge run is started so that the ramp up to 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. Refer to Important Considerations noted above.

As a visual check on the effectiveness of centrifugation, 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 1500g x 2 minutes.

Cushions

Cushions supplied with the centrifuge should be used under the wash plates at all times. This will help prevent cracking of the wash plates. Wash plates typically may be used to process over 100 **CENTRI•SEP 96** plates. If your wash plates are cracking, check the fit of the plate on the carrier. The plates should sit evenly on the carrier cushion, and tightly enough so that the plates cannot move around on the carrier.

g-force

The speed setting required for each centrifuge to reach 1500 x g will vary with the radius of the rotor used. The centrifuge manufacturer usually supplies a table or nomogram relating rpm to g force. Alternatively, the table at the bottom of the page may be used. Values for fractional radii (i.e., 9.5cm) may be determined by interpolation.

Incompatible Centrifuges

The CENTRI·SEP 96 filter plate/wash plate combination will not fit on the Heraeus Megafuge (possibly sold under Baxter name as well). There is insufficient clearance between the carrier trunnion and spindle.

Manual Sample Application

CENTRI•SEP 96 plates are manufactured using precision filling equipment. This method ensures the extremely uniform gel bed heights required for robotic sample application. Since many users will be loading samples with multi-channel pipettors rather than robots, the following practices should be followed.

1. Samples should be loaded onto the center of the matrix bed, without touching the pipet tips to the bed.

2. Allow the sample to "touch-off" onto the gel bed rather than "blowing-out" the pipette tips.

3. Place the forefinger of your non-pipetting hand alongside the plate row to which the samples are to be applied. Rest the pipet tips on this finger as they are being guided to the center of the gel beds.

Storage

Store unused plates at 2-8 degrees C. **Plates must be equilibrated to room temperature prior to use.**

TABLE I. Relationship between Rotor radius and rpm required to achieve 1500 x g.

Radius (cm)	7	8	9	10	11	12	13	14
Rpm required to reach 1500 x g	4375	4093	3860	3660	3490	3342	3211	3094

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