

CENTRI•SEP 96 PLATES

For Research Use Only

PRINCIPLE

CENTRI•SEP 96 plates are recommended by Applied Biosystems, Inc for fast, efficient removal of excess dye terminators from completed DNA sequencing reactions. The plates are also useful for removing excess nucleotides, small primers, radioactive and fluorescent precursors, salts and buffers.

CENTRI•SEP 96 plates are pre-packed with a hydrated, cross-linked gel suitable for removing excess terminators and nucleotides from dye terminator sequencing reaction mixtures. The plates are sealed top and bottom with an adhesive foil seal to prevent drying of the gel beds.

Purified reaction products from CENTRI•SEP 96 plates are collected into a standard 96 well PCR plate. The purified reaction mixtures are suitable for sequencing on an automated DNA sequencer such as the ABI 3100, ABI 3700, ABI 3130 and 3130x1, ABI 3730 and 3730x1. Subsequent drying and denaturing steps may be accomplished in Savant Speed-Vac or thermal cycler devices.

PROCEDURAL NOTES

Most centrifuges, either bench or floor models, which accept micro-plate rotors may be used with the CENTRI-SEP 96 protocol. However, the rotor must accept a plate stack approximately 5.1 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.

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 1500 x g

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 1500 x g for 2 minutes.

Cushions:

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

g-Force:

Speed settings 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 following table may be used. Values for fractional radii (i.e., 9.5 cm) may be determined by interpolation.

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

If you are not sure of the g-force generated by your centrifuge at specific speeds, you can 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 = 1500
 r = 7.5 cm:

$$rpm = \sqrt{\frac{1500}{(1.119 \times 10^{-5})(7.5)}} = 4226$$

Manual Sample Application:

CENTR-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 steps should be followed:

1. Samples should be loaded in the centers of the matrix beds, without touching the pipette tips to the beds or walls of the well
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
4. Rest the pipette tips on this finger as they are being guided to the center of the gel beds

STORAGE AND STABILITY

The CENTRI-SEP 96 plates are stable until the indicated expiration date when stored at 2-8°C.

MATERIALS PROVIDED

CATALOG NO.	CONTENTS
CS-961	96-well Plates (2) 96-well PCR Collection Plates (2) Thermal Seal® Film (2)
CS-962	96-well Reusable Wash Plates (2)
CS-963	96-well Plates (25)
CS-965	96-well Plates (50)

ADDITIONAL MATERIALS RECOMMENDED

- 2 Reusable 96-well wash plates (CS-962; supplied on request for use with CS-961)
- 96-well collection plates for CS-963, CS-965
- Thermal Seal[®] Films for CS-963, CS-965
- Any centrifuge with rotor and microwell plate carrier capable of handling stacked plates (5.1 cm height) at 1500 x g. Plates can also be centrifuged in vacuum concentrators, such as Savant Speed-Vac rotors like DSR6, MPTR-8-210, PRO-20 System and Genevac EZ-2.
- Multi-channel pipettor and tips

<u>CATALOG NO.</u>	<u>SIZE</u>
CS-961	2 pack
CS-963	25 pack
CS-965	50 pack

CENTRI-SEP 96 Protocol

CENTRI•SEP 96 plates must be allowed to equilibrate to room temperature before use. We recommend that the plates be removed from the refrigerator at the same time the sequencing reactions are initiated. This will allow sufficient time for the plates to warm.

1. Remove the adhesive foil from the bottom and then from the top of the CENTRI-SEP 96 plate.
2. Stack the CENTRI-SEP 96 plate on top of a 96-well wash plate and centrifuge at 1500 x g for 2 minutes. Use an external timer and start timing when the rotor has reached the set speed. Discard the liquid in the wash plate. The gel matrix in the wells should appear opaque at this point.
3. Transfer the samples (20 μ L or less) to the individual wells in the CENTRI-SEP 96 plate, taking care to place the samples in the centers of the gel beds.
4. Stack the CENTRI-SEP 96 plate on top of a 96-well collection plate and centrifuge at 1500 x g for 2 minutes.
5. Remove the 96-well collection plate containing the cleaned samples and dry in a speed-vac equipped with the appropriate rotor. Alternatively the plate can be sealed for storage.