AB-0952 / v2 / 0208

Separation Plate

Description: The Separation Plate is a 96-well plate containing polyethylene frits with a pore size of 30–45µm. It can be used in combination with a suitable matrix for gel filtration applications, including dye terminator removal.

Ordering Information:

AB-0952	Separation Plate	24 plates

Applications:

Preparation of gel filtration matrix for dye terminator removal

1. Hydrate 5g of a suitable gel filtration matrix (e.g. Sephadex™ G-50 from Amersham Biosciences) in 70ml sterile ddH_2O in a centrifuge bottle. 5g will swell to approximately 50ml. Allow 30 minutes for complete swelling.

2. Centrifuge at 1,600rpm (750 x g) for 3 minutes with 0 deceleration. Pour off supernatant. Add ddH_2O to 70ml, shake well and repeat spin. Wash twice more with ddH_2O.

3. Wash once with a 20% ethanol solution. Remove ethanol supernatant and resuspend in an excess of 20% ethanol.

Dispensing of gel filtration matrix solution

1. Prior to aliquoting, resuspend matrix/ethanol solution using a magnetic stirrer. Seal the bottom of the Separation Plate with a strong adhesive seal (AB-0558 recommended).

2. Pipette 520µl of matrix suspension into each well of the Separation Plate. Make sure that the solution is thoroughly resuspended throughout dispensing.

3. Seal the top of the Separation Plate with a heat or adhesive seal. Use within 24 hours.*

*Thermo Fisher Scientific also supply a Dye Terminator Removal Kit (AB-0943) which comes pre-prepared with Sephadex and can be stored at ambient temperature for up to six months.

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Protocol for dye terminator removal

1. Remove the sealing material from the top and bottom of the Separation Plate. Keep the plate horizontal at all times.

2. Place the Separation Plate onto a wash plate and centrifuge for 3 minutes at 950 x g to remove the storage buffer. Discard the flow-through.

3. Place the Separation Plate onto a sample plate (e.g. AB-0600).

4. Add sequencing reaction mix (typically 20µl) to the centre of the well. If the volume of samples is lower than 20µl, adjust to 20µl with water. **Avoid disturbing the matrix or allowing the mixture to run down the edge of the wells.**

5. Centrifuge plates for 3 minutes at 950 x g.

6. Prepare the eluted samples according to the instructions provided with the DNA sequencer.

Troubleshooting:

Carryover of dye terminators into sequencing profile

The sample volume should be no more than 20µl. The sample should be pipetted directly onto the centre of the matrix bed and it should be applied as slowly as possible to avoid liquid flowing down the sides of the well.

Low signal intensity

For low volume samples, vacuum dry the eluate and resuspend in 10µl loading buffer for capillary sequencers or 4µl of formamide loading dye for slab-gel sequencers.

Suggested Loading Conditions:

Capillary Sequencers

Injection conditions: 3 kV for 75 seconds
Electrophoresis conditions: 8kV for 120 minutes

Slab-gel Sequencers:

Load 1.5–2.0µl of resuspended sample in each well of a sequencing gel.

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