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ProductInformation

SigmaSpin™ Sequencing Reaction Clean-Up, 96-well post-reaction clean-up plates

Catalog Numbers **S4309, S4434, and S4559**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

SigmaSpin™ Post-Reaction Clean-Up Plates provide a convenient method for removing small molecules including unincorporated dyes/nucleotides and salts from sequencing reactions. BigDye™ v3.1 reactions require a modified procedure for optimal performance. The plates are also useful for post-PCR† desalting and primer removal.

The plates are packed with a size exclusion matrix suspended in water containing 25 ppm of Kathon® (a preservative). The gel matrix has been selectively optimized to absorb small molecules while maximizing recovery of single and double stranded DNA greater than 20 base pairs.

Components Provided	Catalog Number	Number of Plates		
		S4309	S4434	S4559
96 well SigmaSpin Post-Reaction Clean-Up plate (gel filtration plates)	S4559	2	10	50
48 well wash plates	P4736	2	10	0*
96 well collection plates	P2068	2	10	0*

**The package of 50 plates (S4559) does not include wash or collection plates.*

Equipment and Reagents Required But Not Provided

- Centrifuge capable of 750 x *g* with appropriate 96 well plate rotor
- Multichannel pipettor
- Vacuum centrifuge with 96 well plate capabilities or a lyophilizer to dry the eluate

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

The gel filtration plates may be stored for 1 month at room temperature or for up to 1 year at 2–8 °C.

Procedure

With the exception of BigDye v3.1, the following procedure needs no modifications for dye terminator and primer chemistries. Each plate cell is sufficient for purification of one reaction.
Note: For best results, the timing in steps 4 and 7 should begin when the rotor achieves the recommended speed.

1. Bring the SigmaSpin Plate to room temperature prior to use if stored at 2–8 °C.
2. With the sealing mats in place, gently tap the plate on a bench surface to restore the resin to the bottom of the plate.
3. Remove the top and bottom sealing mats and stack the SigmaSpin Plate on a 48 well wash plate. Verify that the alignment is such that two columns empty into one well of the wash plate.
4. Place the above assembly in the centrifuge rotor with the Sigma logo facing the center. Centrifuge at 750 × *g* for 2 minutes. The centrifuge speed (*RPM*) to achieve a force of 750 × *g*, can be estimated from the enclosed scale or calculated from the following equation:

$$RPM = 8190 \times \sqrt{1/r}$$

RPM = speed (revolutions per minute)
r = rotor radius (in centimeters)

5. Discard the eluate.
6. Stack the SigmaSpin Post-Reaction Cleanup Plate on a 96 well collection plate. Verify that the two plates are properly aligned. Pipette the sample solution directly into the center of each well.

7. Place the above assembly into the centrifuge rotor with the Sigma logo again facing the center of the rotor. Spin at 750 x *g* for 4 minutes. Retain the eluate.
8. The eluted DNA is in water containing ~25 ppm preservative. For some applications it may be possible to use the DNA without further manipulations. For sequencing it is common to dry the samples in a lyophilizer, vacuum desiccator, or a rotary evaporator designed for 96 well plates, followed by dissolution in an appropriate loading buffer and electrophoretic separation.

Modifications for BigDye v3.1 reactions

BigDye v3.1 Terminators have been shown to aggregate and reduce the effectiveness of spin plate purification. The following methods disrupt dye aggregation and allow typical purification.

A. – Use of SeqSaver™ Sequencing Premix Dilution Buffer (Catalog Number S3938)

Dilution of sequencing reaction premix with SeqSaver, according to recommended protocols, yields optimal results when used with SigmaSpin plates.

1. Dilute BigDye v3.1 reaction premix with an equal volume of SeqSaver (1:1) and perform the sequencing reaction as usual. It is possible to optimize different templates so that BigDye v3.1 may be further diluted. Then follow the SigmaSpin procedure.

B. – Use of SDS

Alternatively, the addition of SDS followed by heating just prior to desalting can be performed (see ABI user bulletin 4330951).

1. Add SDS to the completed sequencing reaction to a final concentration of 0.2% (add 1 volume of 2.2% SDS solution to 10 volumes of the sequencing reaction).
2. Heat the samples at 98 °C for 5 minutes.
3. Cool the tubes to 25 °C for 10 minutes.
4. Follow the SigmaSpin procedure.

Preparation of 1 ml of 2.2% SDS solution:

a. Dilute 220 µl of 10% SDS (Catalog Number L4522) with 780 µl of water (Catalog Number W4502) **OR**
b. Dissolve 22 mg of SDS (Catalog Number L4390) in 1 ml of water (Catalog Number W4502).

Troubleshooting Guide

Problem	Cause	Solution
Sequence quality is poor or signal strength is low.	Template and/or primer(s) are of low quality.	Check purity of template and primer(s) and repurify template and primer(s) if necessary.
	Improper cycle sequencing conditions	Optimize cycle sequencing parameters.
	Errors including • Improper loading of gel filtration well • Inaccurate centrifugation speed • Inaccurate centrifugation time	Follow recommended procedures carefully.
Unincorporated dye terminators are incompletely removed.	Sample not loaded directly into the center of the well.	Carefully load sample directly into the center of each well.
	The gel filtration matrix was overloaded.	Reduce the quantity of the reaction loaded.

Related Products	Catalog Number
SeqSaver Sequencing Premix Dilution Buffer	S3938
SigmaSpin Post-Reaction Clean-Up Columns	S5059
Capillary Electrophoresis Running Buffer (10×) for automated DNA sequencing	B4930
Tris-Borate-EDTA (TBE) Buffer, 10× Concentrate	T4415

Related Book	Catalog Number
DNA Sequencing Strategies, Ansorge, W., Voss, H. and Zimmerman, J., Wiley-Liss, New York, NY, 1996, 208 pp., soft cover.	Z373850

Reference

1. Krakowski, K., et al., Rapid Purification of Fluorescent Dye-Labeled Products in a 96 well Format for High-Throughput Automated DNA Sequencing. Nucleic Acid Res., **23**, 4930-4931 (1995).

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.
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BigDye is a trademark of Applied Biosystems.
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