3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

SigmaSpin™ Sequencing Reaction Clean-Up, Post-Reaction Clean-Up Columns

Catalog Number **S5059**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

SigmaSpin Post-Reaction Clean-Up Columns 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 columns are also useful for post-PCR† desalting and primer removal. They are packed with a size exclusion matrix suspended in water that contains 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. This kit is sufficient for purifying 70 sequencing reactions.

Reagents Provided	Catalog Number	Number of Items
SigmaSpin Columns	S0185	70
Collection Tubes	T 7813 or T5449	140

Equipment Required But Not Provided

- Variable speed desk-top microcentrifuge
- Vacuum centrifuge 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 columns 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 standard dye terminator and primer chemistries.

Note: For best results, the timing in steps 2 and 6 should begin when the rotor achieves the recommended speed.

1. Loosen the cap by a half turn, then snap off the bottom closure.

2. Place the column into one of the collection tubes and centrifuge at 750 x *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)

3. Discard the eluate and the collection tube.
4. Place the column in a new collection or microcentrifuge tube.
5. Pipette the sample solution directly into the center of the column.
6. Place the above assembly into the centrifuge rotor and spin for 4 minutes at 750 x *g*.
7. Discard the column and 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 rotary evaporator 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 column 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 columns.

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 column • Inaccurate centrifugation speed • Inaccurate centrifugation time	Follow recommended procedures carefully.
	Sample was not loaded directly into the center of the column.	Carefully load sample directly into the center of the column.
Unincorporated dye terminators are incompletely removed	The column was overloaded.	Reduce the quantity of the reaction loaded.

Related Products	Catalog Number
SeqSaver Sequencing Premix Dilution Buffer	S3938
SigmaSpin Sequencing Reaction Clean-Up, 96-well post-reaction clean-up plates	S4309 S4434 S4559
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

TD,JC,PHC 06/11-1