Pierce™ Dye and Biotin Removal Spin Columns

2-, 5-, and 10-mL columns for processing up to 4 mL sample volumes

Catalog Numbers A44298, A44299, A44300, A44301, A44302, and A44303

Doc. Part No. 2162737 **Pub. No.** MAN0018804 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Thermo Scientific™ Pierce™ Dye and Biotin Removal Spin Columns contain a ready-to-use resin that is uniquely designed for rapid removal of non-conjugated fluorescent dyes, biotin, reducing agents, and crosslinkers with exceptional protein recovery.

Removal of free dye after a labeling reaction is essential for the accurate determination of dye-to-protein ratios. Pierce™ Dye and Biotin Removal Spin Columns can be used to remove most fluorescent dyes. For optimal protein recovery and dye removal, ensure that the appropriate amount of sample and buffer conditions are used.

This product is recommended for the removal of unreacted biotin, fluorescent dyes, crosslinkers, and reducing agents from proteins >7 kDa.

Contents and storage

Product	Cat. No.	Amount	Storage
Pierce™ Dye and Biotin Removal Spin Columns, 2 mL[1]	A44298	5 columns	4°C
	A44299	25 columns	
Pierce™ Dye and Biotin Removal Spin Columns, 5 mL[1]	A44300	5 columns	
	A44301	25 columns	
Pierce™ Dye and Biotin Removal Spin Columns, 10 mL[1]	A44302	5 columns	
	A44303	25 columns	

[1] The resin is supplied in a 0.1 N NaCl/0.05% sodium azide solution.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Variable-speed benchtop microcentrifuge	MLS
Consumables	
15-mL conical tubes (for use with 2-mL and 5-mL spin columns)	MLS
50-mL conical tubes (for use with 10-mL spin columns)	MLS

Procedural guidelines

- Do not reuse the purification resin.
- Limit DMF and other organic solvents to ≤10%.
- Do not use this product for desalting or buffer exchange.

Prepare the spin column

1. Twist to remove the bottom plug of the column, then loosen the cap. Do not remove the cap.
2. Place the column in a 2-mL collection tube, then centrifuge the column-tube assembly at 1,000 × *g* for 2 minutes to remove the storage buffer. Discard the flow-through.
3. Place a mark on the side of the column where the resin is slanted upward. For all subsequent centrifugation steps, place the column in the microcentrifuge with the mark facing away from the rotor center.

   IMPORTANT! Improper orientation of the column during centrifugation can result in reduced small molecule removal.

4. *(Optional)* Add the appropriate volume of equilibration buffer to the column as indicated, then centrifuge at 1,000 × *g* for 2 minutes. Discard the flow-through.
   - **For a 2-mL column**—add 2 mL of equilibration buffer.
   - **For a 5-mL column**—add 5 mL of equilibration buffer.
   - **For a 10-mL column**—add 10 mL of equilibration buffer.

Process the sample

1. Place the prepared column in a new collection tube, then remove the cap.
2. Slowly apply the appropriate volume of sample to the center of the settled resin as indicated.
   - **For a 2-mL column**—add 400–700 µL of sample.
   - **For a 5-mL column**—add 1–2 mL of sample.
   - **For a 10-mL column**—add 2–4 mL of sample.
3. Centrifuge the column-tube assembly at 1,000 × *g* for 2 minutes to collect the sample. Discard the column.

   The sample is in the collection tube.
4. Store the sample protected from light at 4°C for up to one month, or add sodium azide at a final concentration of 0.02% for long-term storage.

   Alternatively, samples can be stored at −20°C in single-use aliquots to avoid freeze/thaw cycles. If the final sample concentration is <1 mg/ml, we recommend adding 1–10 mg/ml of a stabilizing agent, such as bovine serum albumin.

Troubleshooting

Observation	Possible cause	Recommended action
Sample or buffer does not flow through the resin	A problem occurred during centrifugation.	Ensure that the centrifuge is in proper working condition.
		Ensure that the bottom plug of the column is removed and the top cap is loosened.
Insufficient removal of free dye	Low resin-to-free dye ratio.	Repeat the procedure using a higher resin-to-free dye ratio.
Sample contains contaminants	The sample was not loaded on the column properly.	Add the sample directly to the center of the settled resin, then carefully touch the pipette tip to the resin to expel the entire sample.
		Avoid contact with the sides of the column.
	A problem occurred during centrifugation.	Do not exceed the recommended centrifugation speed or time.

Related products

Product	Cat. No.
Pierce™ Biotin Quantitation Kit	28005
Pierce™ Fluorescence Biotin Quantitation Kit	46610
Pierce™ Rapid Gold BCA Protein Assay Kit	A53225
Slide-A-Lyzer™ G2 Dialysis Cassettes, 20K MWCO, 70 mL, 6 units	87738
EZ-Link™ Sulfo-NHS-SS-Biotin, No-Weigh™ Format	A39258
EZ-Link™ Hydrazide-PEG4-Biotin	21360
Bond-Breaker™ TCEP Solution, Neutral pH	77720
Pierce™ TCEP-HCl, No-Weigh™ Format	A35349
BS3 (bis(sulfosuccinimidyl)suberate), No-Weigh™ Format	A39266
Alexa Fluor™ 660 NHS Ester (Succinimidyl Ester)	A20007
DyLight™ 488 NHS Ester	46402
Pierce™ DTT (Dithiothreitol)	A39255
DTNB (Ellman's Reagent) (5,5-dithio-bis-(2-nitrobenzoic acid)	22582
Pierce™ BCA Protein Assay Kit	23225

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0018804

Revision	Date	Description
A.0	6 August 2019	New document for new product.