

Zeba™ Dye and Biotin Removal Columns

0.5-mL columns for processing 50–120 µL sample volumes

Catalog Numbers A44296S, A44296, and A44297

Doc. Part No. 2162736 Pub. No. MAN0018803 Rev. B.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](https://www.thermofisher.com/support).

Product description

Thermo Scientific™ Zeba™ Dye and Biotin Removal 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. Zeba™ Dye and Biotin Removal 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
Zeba™ Dye and Biotin Removal Columns, 0.5 mL ^[1]	A44296S	5 columns	4°C
	A44296	25 columns	
	A44297	50 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](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Item	Source
Equipment	
Variable-speed benchtop microcentrifuge	MLS
Consumables	
2-mL microcentrifuge tubes	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 400–500 µL of equilibration buffer to the column, then centrifuge at 1,000 × *g* for 2 minutes. Discard the flow-through.

Process the sample

1. Place the prepared column in a new collection tube, then remove the cap.
2. Slowly apply 50–120 µL of sample to the center of the settled resin.
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.
	A problem occurred during centrifugation.	Avoid contact with the sides of the column. 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

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Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0018803

Revision	Date	Description
B.0	29 October 2020	Changing product name to Zeba™ Dye and Biotin Removal Columns.
A.0	6 August 2019	New document for new product.

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