INSTRUCTIONS

Fluorescent Dye Removal Columns

22858 2033.1

Number	Description
22858	**Fluorescent Dye Removal Columns,** contains sufficient materials to process ~10 samples of 250 µl

Kit Contents:

Dye Removal Resin, 5 ml

Spin Columns, 10 each

Microcentrifuge Collection Tubes, 20 each

Storage: Upon receipt store Purification Resin at 4°C. Product shipped at ambient temperature.

Introduction

The Fluorescent Dye Removal Columns enable fast and efficient removal of non-reacted fluorescent dyes from protein labeling reactions. Removing free dye after a labeling reaction is often difficult and time-consuming but is essential for accurate determination of dye-to-protein ratios. The Dye Removal Resin in this kit is highly specialized to produce exceptional protein recoveries while effectively removing non-conjugated dye. Using the appropriate amount of resin and buffer conditions, almost any fluorescent dye can be removed with this kit.

Important Product Information

- Purification resin is for one-time use only (resin cannot be re-used or regenerated).
- Purification resin is not compatible with hydrazide-containing fluorophores.
- Limit DMF and other organic solvents to ≤ 10%.
- Dyes in the green spectral region (e.g., DyLight 549 NHS or NHS-Rhodamine) might require double processing.
- The smallest protein effectively tested with the Fluorescent Dye Removal Columns was aprotinin (6 kDa).

Procedure for Dye Removal

A. Protein Purification

1. For Dye Removal Resin compatibility, adjust the sample pH to within 6.5 to 8.5 and the NaCl concentration to 150 mM.
2. Place a Spin Column into a Microcentrifuge Collection Tube.
3. Mix the Dye Removal Resin to ensure a uniform suspension. Add the resin to the Spin Column. The amount of resin to use depends on the labeling reaction parameters. For example, use 250-400 µl of resin for a 250 µl labeling reaction containing 1-2.5 mg/ml protein labeled with the following dyes at the indicated molar excesses:

Dye	Molar Excess
DyLight 405 Fluor	5-10
DyLight 488 Fluor	10-15
DyLight 549 Fluor	10-15
DyLight 633 Fluor	5-10
DyLight 649 Fluor	5-7
DyLight 680 Fluor	5-8
DyLight 800 Fluor	5-8
FITC	5-10
NHS-Fluorescein	5-10
NHS-Rhodamine	5-7

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Note: Volumes greater than 300 µl or labeling reactions with a high molar excess will require using multiple columns.

4. Centrifuge the column for 30 seconds at 1,000 × *g* to remove the storage solution. Discard the used Collection Tube and place the column into a new tube.
5. Add 100-250 µl of the labeling reaction (see the Important Product Information Section) to the resin in the Spin Column and mix by briefly vortexing.
6. Centrifuge column for 30 seconds at 1,000 × *g* to collect the purified protein.
7. Discard the used column and resin.
8. Store the labeled protein protected from light at 4°C for up to one month (for long-term storage, add sodium azide at a final concentration of 0.02%). Alternatively, store labeled protein in single-use aliquots at -20°C. Avoid repeated freeze/thaw cycles. If the final concentration of conjugate is < 1 mg/ml, add a stabilizing agent, such as bovine serum albumin, at 1-10 mg/ml.

Troubleshooting

Problem	Possible Cause	Solution
Insufficient removal of free dye	Low resin-to-free dye ratio	Process the sample again using a new column and resin
Low protein recovery	Used too much resin	Use an appropriate volume of resin for the specific labeling reaction (see Step 3)

Additional Information

Please visit our website for additional information relating to this product including the following:

- Tech Tip: Calculate Dye:Protein (F/P) Molar Ratios
- Tech Tip: Protein Stability and Storage
- Tech Tip: Extinction Coefficients Guide
- Tech Tip: Convert Between Times Gravity (× *g*) and Centrifuge Rotor Speed (RPM)

Related Products

28384	**BupH™ Borate Buffer Packs,** 40 packs, each pack yields 500 ml
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs, each pack yields 500 ml
46400	**DyLight 405 NHS Ester,** 1 mg
46401	**DyLight 405 NHS Ester,** 5 × 50 µg
46402	**DyLight 488 NHS Ester,** 1 mg
46403	**DyLight 488 NHS Ester,** 5 × 50 µg
46407	**DyLight 549 NHS Ester,** 1 mg
46408	**DyLight 549 NHS Ester,** 5 × 50 µg
46414	**DyLight 633 NHS Ester,** 1 mg
46417	**DyLight 633 NHS Ester,** 5 × 50 µg
46415	**DyLight 649 NHS Ester,** 1 mg
46416	**DyLight 649 NHS Ester,** 5 × 50 µg
46418	**DyLight 680 NHS Ester,** 1 mg
46419	**DyLight 680 NHS Ester,** 5 × 50 µg
46421	**DyLight 800 NHS Ester,** 1 mg
46422	**DyLight 800 NHS Ester,** 5 × 50 µg
46600	**DyLight 405 Maleimide,** 1 mg

46602	**DyLight 488 Maleimide,** 1 mg
46607	**DyLight 549 Maleimide,** 1 mg
46613	**DyLight 633 Maleimide,** 1 mg
46615	**DyLight 649 Maleimide,** 1 mg
46618	**DyLight 680 Maleimide,** 1 mg
46621	**DyLight 800 Maleimide,** 1 mg

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.