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Rapid & Efficient Removal of Unreacted Small Molecules in a Convenient Spin-and-Go Format

Deven Etnyre, Ramesh Ganapathy, Marie Nlend, Joanna Geddes and Chris Etienne, Thermo Fisher Scientific, 3747 N. Meridian Rd., Rockford, IL, United States, 61101

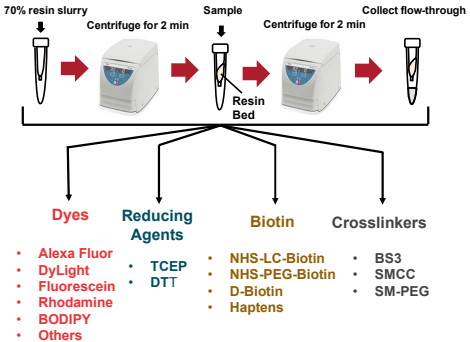
ABSTRACT #2851

Removal of unreacted or residual compounds in the sample-preparation workflow is a key success factor for downstream analysis and processing of biomolecules. For example, during sample preparation of biomolecules such as proteins or nucleic acids, there is often a need to label the biomolecules with dyes, affinity tags, radioactive labels, and mass tags. During these treatments, some amount of the labeling or chemical agent remains in the sample as an unreacted label/chemical. These unreacted small molecules can cause several issues during downstream analysis or use of the biomolecule. For example, free unreacted fluorescent dyes after bioconjugation of protein cause non-specific binding of free-dye resulting in high background issues during fluorescent imaging. Commonly used clean-up methods following sample preparation, such as dialysis, are tedious and lengthy with multiple buffer exchanges sometime resulting in loss of protein due to aggregation. Alternatively, size-exclusion chromatography requires expensive instrumentation in addition to lengthy set up time.

We have developed the Thermo Scientific™ Pierce™ Dye and Biotin Removal Spin Columns, a novel resin that is packed in an easy to use "Spin and Go" format of varying spin column sizes (0.5 mL, 2 mL, 5 mL and 10 mL) and 96-well filter plate to accommodate a range of sample volumes (100 uL - 4 mL). The resin is highly specialized to produce exceptional protein recovery and can be used effectively to remove 4 different classes of small molecules: non-conjugated fluorescent dyes, biotinylation reagents, reducing agents, and crosslinkers. It is a multimodal resin with size exclusion properties and proprietary surface chemistry that allows removal of the above-mentioned small molecules, while the size exclusion property allows for the biomolecule to be efficiently recovered. The improved cleanup efficiency, versatility and protein recovery with this new resin will give researchers ideal post-reaction clean-up of samples allowing for improved results of downstream applications.

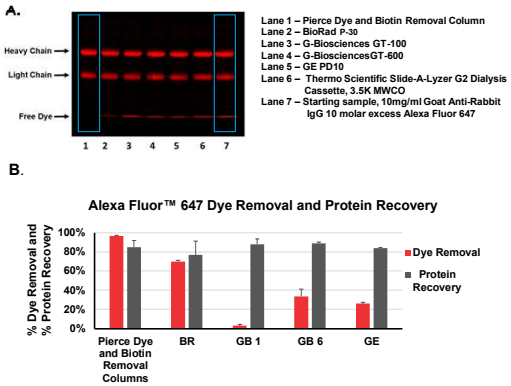
INTRODUCTION

Figure 1. Sample Clean-up Protocol



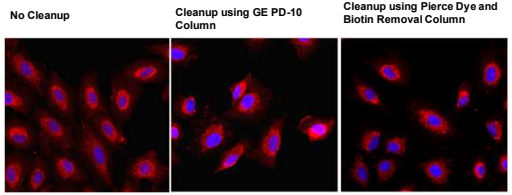
RESULTS

Figure 2. Better Dye Removal Performance



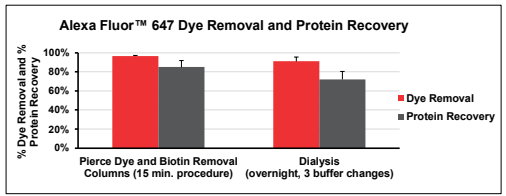
Pierce Dye and Biotin Removal Columns (Product # A44296) provide higher dye removal with excellent protein recovery compared to products from other suppliers (BR = BioRad P-30, GB 1 = G Bioscience GT-100, GB 6 = G Bioscience GT-600, GE = PD10) . Pierce Dye and Biotin Removal Columns (0.5 mL) and alternative products were used to remove free Alexa Fluor 647 Dye from 100 ul samples of 10 mg/mL Goat Anti-Rabbit IgG labeled with 10 molar excess Alexa Fluor 647. Equal volume of sample from each flow through and starting sample (Lane 7) were loaded in the gel. iBright Analysis Software was used to quantitate free dye removal after samples were run on electrophoresis gel and imaged on iBright FL1500 Imaging System (Product # A44241).

Figure 3. Improved Immunofluorescence Results



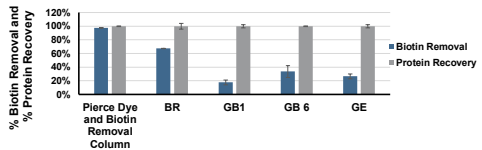
Immunofluorescent staining with PMP70 Alexa Fluor 647 antibody conjugate cleaned up with Pierce Dye and Biotin Removal Columns exhibit lack of nonspecific binding in background. PMP70 polyclonal antibody (Product # PA1-650) was labeled with Alexa Fluor 647 (Product # A20006) and then purified from unreacted dye using Thermo Scientific Pierce Dye and Biotin Removal Columns (Product # A44296). Immunofluorescent analysis of PMP70 (red) in A549 cells. Cells were fixed with 4% Paraformaldehyde in PBS for 15minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and blocked with 1% BSA in PBS. Cells were stained with a PMP70 Monoclonal Antibody, Alexa Fluor 647conjugate with and without cleanup of unreacted dye at a dilution of 2.5 ug/ml in blocking buffer for 1 hour at room temperature protected from light. Nuclei (blue) were stained with Hoechst Dye (Product # 62249) at a dilution of 10,000 in blocking buffer.

Figure 4. Faster Sample Processing with Better Results



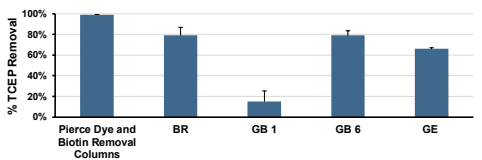
Pierce Dye and Biotin Removal Columns provide higher dye removal with excellent protein recovery compared to dialysis. Pierce Dye and Biotin Removal Columns (Product # A44296) and Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassettes (Product # 87734) were used to remove free Alexa Fluor 647 Dye from samples of 10mg/mL Goat Anti-Rabbit IgG labeled with Alexa Fluor 647 (10 molar excess). Protein recovery was assessed by A280 measurements of starting sample and flow through after dye removal. iBright Analysis Software was used to quantitate free dye removal after samples were run on electrophoresis gel and imaged on iBright FL1500 Imaging System (Product # A44241).

Figure 5. Better Small Molecule Removal



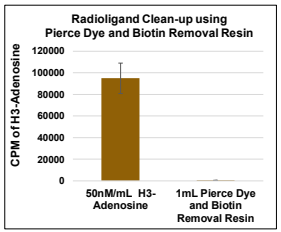
Pierce Dye and Biotin Removal Columns provide higher Biotin removal with excellent protein recovery compared to alternative products. Pierce Dye and Biotin Removal Columns (0.5 mL) and alternative products were used to remove free NHS-LC-Biotin (0.27mM) from 100 ul samples. Protein recovery was assessed by Pierce Rapid Gold BCA (Product # A53227) of starting sample and flow through after dye removal. Pierce Biotin Quantitation Kit (Product # 28005) was used to quantitate free biotin removal.

Figure 6. Greater Reducing Agent Removal Efficiency



Pierce Dye and Biotin Removal Columns provide higher reducing agent removal compared to alternative products. Pierce Dye and Biotin Removal Columns (Product # A44298) and similar products from other suppliers (BR = BioRad P-30, GB 1 = G Bioscience GT-100, GB 6 = G Bioscience GT-600, GE = PD10) were used to remove TCEP (Product # 77720) from of 1mg/mL goat anti-rabbit IgG containing 25mM TCEP in PBS. Reducing agent removal was performed by applying 700 ul of sample to 2 mL columns. Quantification of TCEP removal from flow through compared to starting sample was performed using Ellman's Assay.

Figure 7. Excellent Radioligand Clean-up



Results:

- ✓ A 99.6% decrease in activity is seen when comparing the CPMs in the start solution to the CPMs in the start solution after clean up with resin.
- ✓ Pierce Dye and Biotin Removal Column sufficiently removes free radioligand.

Pierce Dye and Biotin Removal Columns provide efficient radioligand removal. Pierce Dye and Biotin Removal Columns (Product # A44298) were used to remove free radioligand from 140ul 50nM/mL H3-Adenosine. 120 ul of flow through or starting sample was added to 2 mL of scintillation fluid. Samples were read on a scintillation counter to determine counts per minute (CPM). A 99.6% decrease in activity was seen when comparing the CPMs in the start solution versus the CPMs in the sample

CONCLUSIONS

- The Pierce Dye and Biotin Removal Columns enable fast and efficient removal of non-reacted fluorescent dyes, biotin, reducing agents, crosslinkers and radioligands from protein samples.
- Protein recovery and function is maintained post cleanup with no dilution of sample.
- Our results show that the efficiency and binding capacity for removal of small molecules using this resin is better than any resin in the market.

Product	Cat. No.	Amount
Pierce Dye and Biotin Removal Spin Columns, 0.5 mL	A44296S	5
	A44296	25
	A44297	50
Pierce Dye and Biotin Removal Spin Columns, 2 mL	A44298	5
	A44299	25
Pierce Dye and Biotin Removal Spin Columns, 5 mL	A44300	5
	A44301	25
Pierce Dye and Biotin Removal Spin Columns, 10 mL	A44302	5
	A44303	25
Pierce Dye and Biotin Removal Filter Plates, 96 well	A44304	2
	A44305	4

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