Remove detergent from protein samples

TR0019.2

Introduction

Proteins that are bound strongly to the hydrophobic portion of cell membranes require detergents to facilitate dissociation. Because detergents can interfere with many downstream applications, detergent removal may be necessary after initially using them to extract, purify and solubilize proteins. Several different detergent removal methods are available: direct binding, gel filtration, dialysis, precipitation and ion-exchange chromatography. The most appropriate method depends on the effective molecular weight, concentration and other properties of the detergent. This document discusses different detergents and the options available for removing them.

Overview of Removal Methods

Thermo Scientific Pierce® Detergent Removal Resin and Columns enable removal of commonly used ionic, non-ionic and zwitterionic detergents. The Resin removes detergents with greater than 95% efficiency at high concentrations (1 to 5%), while providing exceptionally high protein/peptide recovery. These products are suitable for processing samples for downstream HPLC and mass spectrometry applications. Detergent removal efficiency is described in Table 1.

Table 1. Detergent removal efficiency and protein recovery. Samples (0.1mL) containing 1mg/mL of BSA and detergent were processed through 0.5mL of Pierce Detergent Removal Resin as described in the protocol. Detergent concentrations were measured by various methods. Protein concentration was determined with the Thermo Scientific Pierce BCA Protein Assay.

Detergent	Starting Concentration (%)	Detergent Removal (%)	BSA Recovery (%)
SDS	2.5	99	95
Sodium deoxycholate	5	99	100
CHAPS	3	99	90
Octyl glucoside	5	99	90
Octyl thioglucoside	5	99	95
Lauryl maltoside	1	98	99
Triton* X-100	2	99	87
Triton X-114	2	95	100
NP-40	1	95	91
Brij*-35	1	99	97
Tween*-20	0.25	99	87

Gel filtration removes detergents by size exclusion. Detergent monomers and micelles that are smaller than the filtration-resin pore size (i.e., molecular-weight cutoff, MWCO) can be separated from proteins and other macromolecules that are much larger. We offer Thermo Scientific Zeba™ Desalt Spin Columns, which have a 7000 MWCO.

Dialysis removes detergents by size exclusion but takes more time than gel filtration. Thermo Scientific Slide-A-Lyzer® Dialysis Cassettes are available with different molecular weight cutoff membranes (e.g., 2K, 10K, 20K); depending the relative sizes of the detergent and proteins of interest, the detergent can be separated from the protein by passive diffusion with these units (see Table 2 on the next page).

Thermo Scientific SDS-Out™ SDS Precipitation Reagent and Kit is a quick, convenient precipitation method for removing SDS, a commonly used anionic detergent.

Ion-exchange chromatography will remove nonionic and zwitterionic detergents. In this method, the protein is adsorbed on the resin and the detergent micelles pass through. Changing either the ionic strength or the pH can then elute the protein. Specific binding and elution procedures must be determined empirically for each protein being purified in this manner.

General Detergent Properties

Because the physical properties of detergents affect how easily they can be removed from a sample, these properties must be understood before determining if certain removal methods are appropriate. Micelles are associations of many detergent monomers that form spontaneously in solution. The critical micelle concentration (CMC) of a detergent is the minimum concentration at which micelles form; above the CMC, a detergent exists a in a large molecular weight association. The CMC is also an indicator of the strength at which detergent binds to protein; i.e., low values indicate strong binding and high values indicate weak binding. The CMC is also an indication of a detergent's hydrophilicity. For most detergent to be removed by dialysis or gel filtration, the detergent concentration must be less than the CMC because only detergent monomers can be removed by these methods. A few detergents (e.g., CHAPS and Octyl-ß-Glucoside) have low molecular weight micelles (<10,000) and may be removed by dialysis or gel filtration even when the CMC has been exceeded.

Table 2 indicates the detergent concentrations that can be removed by dialysis or desalting. When detergent removal by size exclusion is desired, choose a detergent with a high CMC and a low micelle molecular weight (e.g., Octyl-ß-Glucoside). Conversely, detergents with a low CMC and a high molecular weight (e.g., Triton X-100) are very difficult to remove from solution.

Table 1. Properties of common detergents.

Detergent	Type	MW	Aggregation Number †	Micelle MW	CMC (mM)	CMC (% w/v)	Cloud Point (°C)	Dialyzable
Triton* X-100	Nonionic	647	140	90,000	0.24	0.0155	64	No
Triton X-114	Nonionic	537	-	-	0.21	0.0113	23	No
NP-40	Nonionic	617	149	90,000	0.29	0.0179	80	No
Brij*-35	Nonionic	1225	40	49,000	0.09	0.1103	>100	No
Brij-58	Nonionic	1120	70	82,000	0.077	0.0086	>100	No
Tween*-20	Nonionic	1228	-	-	0.06	0.0074	95	No
Tween-80	Nonionic	1310	60	76,000	0.012	0.0016	-	No
Octyl-ß-Glucoside	Nonionic	292	27	8000	23 to 25	0.67 to 0.73	>100	Yes
OTG	Nonionic	308	-	-	9	0.2772	>100	Yes
SDS	Anionic	288	62	18,000	6 to 8	0.17 to 0.23	>100	Yes
CHAPS	Zwitterionic	615	10	6149	8 to 10	0.49 to 0.62	>100	Yes
CHAPSO	Zwitterionic	631	11	6940	8 to10	0.505	90	Yes

† The aggregation number in micelles has not been determined for Triton X-114, Tween-20 and Octyl-ß-Thioglucopyranoside

Appropriate Methods of Removal for Specific Detergents

Triton X-100, Triton X-114 and NP-40 detergents are used to solubilize membrane proteins under non-denaturing conditions. Because these detergents have low CMCs, they are difficult to remove by dialysis or gel filtration, but can easily be removed using Thermo Scientific Pierce Detergent Removal Resin at concentrations up to 2%. Another reportedly successful method is to dialyze against a solution containing CHAPS or other detergent that has a small micelle size. As CHAPS diffuses into the sample, it forms mixed micelles with the Triton X-114, thereby decreasing the original micelle size and facilitating its dialysis.

Brij Detergents have varying lengths of a polyoxyethylene chain attached to a hydrophobic chain. Brij-58 is a cetyl ether (C16), and Brij-35 is a lauryl ether (C12). Brij-35 is commonly used in high-performance liquid chromatography (HPLC) applications and to prevent nonspecific binding to gel filtration and affinity chromatography supports. Brij-58 has been used in incubation buffers for nick translation of ribonucleotides or deoxyribonucleoside triphosphates. Brij Detergents are difficult to remove from solution by dialysis, but can easily be removed using Thermo Scientific Pierce Detergent Removal Resin at concentrations up to 1%.

Octyl ß-glucoside and Octyl ß-thioglucopyranoside (OTG) are nondenaturing, nonionic detergents. These detergents have been useful for solubilizing membrane proteins. Because the micelles of these detergents have small molecular weights, they are dialyzed easily from solution even at high concentrations. Dialysis of a solution initially containing 43mM Octyl ß-thioglucopyranoside for 6 hours using 200 volumes of buffer can remove 95% of the detergent. Alternatively they can be removed using Thermo Scientific Pierce Detergent Removal Resin at concentrations up to 5%.

CHAPS and CHAPSO have been used to solubilize intrinsic membrane proteins and receptors and to maintain the functional capability of the protein. These detergents are removed easily by dialysis, gel filtration, ion-exchange chromatography or Thermo Scientific Pierce Detergent Removal Resin at concentrations up to 3%.

Tween-20 and Tween-80 are nondenaturing, nonionic detergents that are polyoxyethylene sorbitan esters of fatty acids. They are used most commonly as blocking agents in biochemical applications and to reduce nonspecific binding to hydrophobic materials. These detergents are difficult to remove from solution by dialysis, but can be removed by some forms of ion-exchange chromatography. Tween 20 will also bind Thermo Scientific Pierce Detergent Removal Resin at concentrations up to 0.25%. Tween 80 has not been tested.

Sodium dodecyl sulfate (SDS) and SDS-Lauryl have a polar anionic sulfate group at one end of their structures and a straight chain nonpolar region at the other end. The dual polarity of SDS allows it to solubilize proteins by imitating their structure. The CMC of SDS is dependent on salt concentration. The CMC for SDS is 8mM in water, 3.5mM in 10mM NaCl, and 1.4mM in 100mM NaCl. Although SDS has a high CMC and a low CMC molecular weight, it tends to bind tightly to cationic molecules because of its anionic nature. Consequently, SDS that is bound to molecules cannot be removed by dialysis. SDS can be removed using Thermo Scientific Pierce Detergent Removal Resin at concentrations up to 2.5%. For small samples, SDS-Out™ SDS Precipitation Reagent is a convenient method for removing excess SDS from solutions. However, it will not remove SDS that is bound to protein.

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.

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