TECHNICAL RESOURCE

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

Remove detergent from protein samples

TR0019.0

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 or purify protein. Several different detergent removal methods are available: gel filtration, dialysis, Extracti-Gel® D Detergent Removing Gel (Product No. 20208, 20346), SDS-Out™ SDS Precipitation Reagent (Product No. 20308, 20310) and ion-exchange chromatography. Which method is appropriate depends on the effective molecular weight, concentration and other properties of the detergent. No one method is appropriate for all situations.

Overview of Removal Methods

Gel filtration (e.g., D-Salt™ Desalting Columns or Zeba™ Micro Desalt Spin Columns) removes detergents by size exclusion. Detergent monomers remain in the internal pores of the gel, and the protein is free to pass through the in the void volume. Pierce offers desalting columns with excellulose, dextran, polyacrylamide, and other matrices.

Dialysis removes detergents by size exclusion but takes more time than gel filtration. Slide-A-Lyzer® Dialysis Cassettes (e.g., Product No. 66382), which can reduce dialysis time and provide excellent sample recovery, are available for several sample volumes and with 10 kD, 7 kD and 3.5 kD molecular weight cutoff membranes. Slide-A-Lyzer MINI Dialysis Units (e.g., Product No. 69576) are also available for dialyzing very small (10-100 µl) sample volumes.

Extracti-Gel® D Detergent Removing Gel and the SDS-Out™ SDS Precipitation Reagent and Kit are quick, convenient methods for removing detergents that cannot be removed by either dialysis or gel filtration. Extracti-Gel® D Detergent Removing Gel is effective for binding and removing milligram quantities of many detergents from protein solutions. The SDS-Out™ Reagent is specific 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 choosing which removal method is 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 1 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.

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. Extracti-Gel® D Detergent Removing Gel works well to remove these detergents from solution.

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 they may be removed by Extracti-Gel® D Detergent Removing Gel.

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 43 mM Octyl ß-Thioglucopyranoside for 6 hours using 200 volumes of buffer can remove 95% of the detergent. Extracti-Gel® D Detergent Removing Gel will also remove these detergents from solution.

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, Extracti-Gel D Detergent Removing Gel or ion-exchange chromatography.

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 Tween®-20 can be removed by Extracti-Gel® D Detergent Removing Gel. Alternatively, these detergents may be removed by ion-exchange chromatography.

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 8.0 mM in water, 3.5 mM in 10 mM NaCl, and 1.4 mM in 100 mM 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. Extracti-Gel® D Detergent Removing Gel has been used successfully to remove nonprotein-bound SDS from solutions. One milliliter of gel can remove up to 80 mg of SDS in a 100 mM phosphate buffer, pH 7.0. For small samples, SDS-Out™ SDS Precipitation Reagent is a convenient method. However, neither of these methods will remove SDS that is bound to protein.

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	8,000	23-25	0.672-0.730	>100	Yes
OTG	Nonionic	308	-	-	9	0.2772	>100	Yes
SDS	Anionic	288	62	18,000	6-8	0.173-0.230	>100	Yes
CHAPS	Zwitterionic	615	10	6,149	8-10	0.492-0.615	>100	Yes
CHAPSO	Zwitterionic	631	11	6,940	8-10	0.505	90	Yes

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

Related Pierce Products

28340 **Surfact-Pak™ Detergent Sampler**, contains a 10 ml ampule of each Surfact-Amps® Purified Detergent or 100 mg solid of each detergent listed in Table 1, except SDS.

28364 **SDS**, 100 g

The most current versions of all product instructions and technical resources are available at *www.piercenet.com*.