INSTRUCTIONS

# OTG
# l-*S*-Octyl-β-D-Thioglucopyranoside (Octylthioglucoside)

28351

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0238

## Octylthioglucoside

The data below are not specifications. They are typical values for this detergent.

**Description:** Nonionic detergent

**MW:** 308.4

**Cloud Point:** > 100°C

**CMC:** 9 mM

**Applications:** solubilizing membrane proteins

## Product Description

| NUMBER | DESCRIPTION |
|---|---|
| **28351** | **OTG, 1-*S*-Octyl-β-D-Thioglucopyranoside (Octylthioglucoside),** *5 g*<br>Supplied as dry powders and should be stored refrigerated away from moisture. |

## Introduction

Biological membranes form large aggregates composed of lipids and proteins and perhaps some covalently bound carbohydrates. To study membrane structure and function it is usually necessary to solubilize the membrane into its components. Proteins which are strongly bound to the hydrophobic portion of the membrane (intrinsic proteins) often require detergents for their dissociation from the membrane.

Detergents are amphipathic structures with an apolar end of aliphatic or aromatic character and a polar end which may be charged or uncharged. They are more hydrophilic than lipids and therefore more water soluble. Detergent molecules allow the dispersion of water insoluble compounds into aqueous media and are usually used to isolate and purify membrane proteins in a native form.

Detergents can be denaturing or nondenaturing. Denaturing detergents can be anionic such as sodium dodecyl sulfate (SDS) or cationic such as ethyl trimethyl ammonium bromide. These detergents totally disrupt the membranes and denature the protein by breaking protein-protein interaction. Nondenaturing detergents can be divided into nonionic detergents such as Triton® X-100, bile salts such as cholate and zwitterionic detergents such as CHAPS. Zwitterionics contain both a cationic and anionic group in the same molecule. The positive electric charge is neutralized by the negative charge on the same or an adjacent molecule.

Detergents in low concentrations will form a monolayer at the air-water interface. However at higher concentrations the monomers form aggregates called micelles (Figure 1). A micelle is a thermodynamically stable colloidal aggregate formed above a narrow concentration range. In micelles the hydrophobic apolar groups of the detergent molecules are sequestered from the water by the polar groups facing outward covering the surface of the micelle.

The critical micelle concentration (CMC) of a detergent is the concentration where micelles form. The critical micelle temperature (CMT) is the lowest temperature at which micelles can be formed. If thetemperature is decreased below the CMT, a crystalline solution is formed. The CMT can thus be described as a sudden clearing of the cloudy crystalline

suspension. The aggregation number is the number of detergent monomers that make up a micelle. Cloud point of a detergent is a method of identifying and checking the quality of detergents, especially nonionic ones. At certain temperatures, the detergent molecules become insoluble and separate from the solution. This is considered the cloud point of that detergent and is caused by a decrease in head group hydration.

Detergent properties are affected by experimental conditions such as temperature, pH, ionic strength, concentration and the presence of various additives. The addition of double bonds or branching of the hydrophobic domain will increase the CMC. A higher CMC occurs with more hydrophilic molecules. Hydrophilic molecules oppose micelle formation due to electrostatic repulsion of the more polar head groups. The CMC of these hydrophilic molecules can be decreased by the addition of a counter ion concentration, thus reducing electrostatic repulsion. The effect of temperature on the CMC is small for ionic detergents but the CMC of nonionic detergents decreases with increasing temperature. An increase in ionic strength results in a dramatic increase in aggregation number, probably due to a decrease in the repulsion between ionic headgroups. An increase in the size of the nonpolar group, as in an elongation of the alkyl chain, increases the hydrophobicity of the surfactant, and CMC decreases as the hydrophobic character increases. An increase in concentration results in larger micelle sizes with ionic surfactants. Additives which disrupt water structure such as urea increase the CMC.[1]

In general, the nonionic detergents will have a high molecular weight micelle and a low CMC. The ionic detergents will have a low molecular weight micelle with a high critical micelle concentration. The shape of a micelle also varies from detergent to detergent. Most ionic and nonionic detergents form ellipsoidal micelles. The bile salts have different properties in that they form aggregates which lie back to back instead of forming micelles (Figure 2).

## Binding of Detergents to Proteins

Denaturing detergents such as SDS bind to membrane and nonmembrane proteins at concentrations below the CMC, i.e. as monomers. The reaction is equilibrium driven until saturated. Therefore, the free concentration of monomers determines the detergent concentration. SDS binding is cooperative (the binding of one molecule of SDS increases the probability that another molecule of SDS will bind to that protein) and alters most proteins into rigid rods whose length is proportional to molecular weight.

Nondenaturing detergents such as Triton® X-100 do not bind to native conformations nor do they have a cooperative binding mechanism. Nondenaturing detergents have rigid and bulky apolar moieties that do not penetrate into water soluble proteins. They bind to the hydrophobic parts of membrane protein. Triton® X-100 and other polyoxyethylene nonionic detergents are inefficient in breaking protein-protein interactions and can cause artifactual aggregations of protein. These detergents will disrupt protein-lipid interactions but are much gentler and thus maintain the native form and functional capabilities of the proteins.

Detergent monomers will bind to water-soluble proteins when both ligand and protein are in low concentration. Therefore no increase in detergent bound to protein is observed after increasing the detergent beyond its CMC. The binding of detergent to proteins competes with the self association of detergent molecules into micelles.[1]

Detergent monomers solubilize intrinsic membrane proteins by partitioning into the membrane bilayer. With increasing amounts of detergents, membranes undergo various stages of solubilization. The initial stage is lysis or rupture of the membrane. At detergent to membrane lipid ratios of 0.1-1 (by weight) the lipid bilayer usually remains intact and selective extraction of membrane protein occurs. Increasing the ratio to 2:1, solubilization of the membrane occurs resulting in mixed micelles. These include phospholipid-detergent micelles, detergent-protein micelles, and lipid-detergent-protein micelles. At a ratio of 10 to 1 an exchange of virtually all the lipid for detergent is seen around the protein. The amount of detergent needed for optimal protein extraction depends on the CMC, aggregation number, temperature, and nature of the membrane and the detergent. The solubilization buffer should contain sufficient detergent to provide greater than 1 micelle to protein molecule to ensure there are not two proteins contained in one micelle.[2]

## Removal of Detergent from Solubilized Proteins

Some proteins are active in a solubilized form, however others may be modified or inactivated due to loss of lipid interaction. To study the function of these proteins it is necessary to reconstitute them into "membranes." The bilayer membrane can be formed by detergent removal in the presence of phospholipids or membrane lipid mixtures. One problem is that the original orientation of the protein in the random membrane reassembly may not be accomplished. Frequently it is necessary to remove detergents from solution. The function of membrane proteins can be studied by reassociation of a

membrane protein in apurified form with an artifactual lipid bilayer by removal of the solubilizing detergents.

Detergent removal can be attempted in a number of ways. Dialysis works fairly well with detergents that have a very high CMC, such as the *n*-octyl glucosides. Dialysis is ineffective for the removal of detergents with a low CMC since most of the detergent molecules will be in micelles and only the monomer can be dialyzed. Ion exchange chromatography can be utilized. The solubilized protein can be applied to an ion exchange chromatography column and the column then washed with buffer minus detergent. The detergent will be removed as a result of the equilibration of the buffer with the detergent solution. Protein can be run through a sucrose density gradient. As the protein sediments through the sucrose gradient the detergent will come off due to chemical potential.

One of the most successful means of removing detergents from protein solutions is by the use of Pierce's Extracti-Gel™ D Detergent Removing Gel (Product Nos. 20208, 20303 and 20346). The Extracti-Gel™ D support is a versatile affinity matrix which allows the small detergent molecules to penetrate into the pores and interact with the immobilized ligand. As the protein solution is passed through the Extracti-Gel™ D column, the detergent binds to the column and free protein elutes in the void volume.

## Solubilization of Lipids

The amount of detergent available for solubilizing lipids is only that detergent present in micelles. For a detergent with a low CMC this is essentially the amount of detergent added, whereas for a detergent with a high CMC it is theoretically the CMC. The concentration of detergent in detergent-lipid mixed micelles will be the difference between the total detergent concentration and its monomer concentration in the mixture.

When a detergent is added to a phospholipid bilayer in an aqueous solution, the detergent penetrates between the bilayers and the solution. Mixed micelles form after the phospholipid bilayers are saturated with detergent. Both the detergent saturated bilayer and the phospholipid saturated mixed micelles will exist until sufficient detergent is added to convert all the bilayers into mixed micelles. As with the solubilization of proteins, the amount of detergent necessary for the formation of mixed micelles is dependent on temperature, ionic strength, pH and the nature of membrane and detergent.[3]

## Use of Multiple Detergents for Some Applications

Often a single detergent is not versatile enough for solubilization and analysis of protein. The protein can be solubilized in one detergent and then replaced with a detergent suitable for analysis of the protein. The protein-detergent micelles formed with the first detergent should be separated from pure detergent micelles. When these are added to an excess of the detergent for analysis, the protein is found in mixed micelles with both detergents. Separation of the detergent-protein micelles can be accomplished with ion exchange or gel filtration chromatography, dialysis or buoyant density.

## Octylthioglucoside

Octylthioglucoside (OTG) is a nondenaturing, nonionic detergent that is very water soluble and is easily dialyzed from solution. This detergent is useful for solubilizing membrane proteins and has low UV absorbance at 280 nm. The hydrophobic region in octylthioglucoside is the *n*-octyl group and the hydrophilic region is the thioglucose. Unlike the Triton® X and Brij® detergents, octylthioglucoside is homogeneous. Octylthioglucoside, with a relatively high CMC of 9 mM, has been used at concentrations of 1.1-1.2% (w/v) to solubilize membrane proteins.[4,5]

Octylthioglucoside was first synthesized to offer an alternative to a similar detergent, octylglucoside. Octylglucoside has a high CMC (25 mM), but is expensive to manufacture and there are some inherent problems with its use in biological systems because it can be hydrolyzed by β-glucosidase.[4] Membrane proteins of *Escherichia coli* were readily solubilized by octylthioglucoside and the detergent was found to be more stable in solution than octylglucoside.4,5 Dialysis of a 43 mM solution of octylthioglucoside versus 50 mM potassium phosphate buffer, pH 7.5 at 4°C removed 95% of the detergent after 6 hours using 200 volumes of buffer.[4] The nonionic nature of this detergent would allow its use in ion exchange chromatography and isoelectric focusing.[5]

Octylthioglucoside is compatible with the BCA* Protein Assay Reagent (Product No. 23225) at a concentration of 3%.

Octylthioglucoside is easily removed from protein solutions by dialysis but other means are possible. Among the methods

used to remove unbound detergent from protein samples are solvent extraction, affinity chromatography, phase partitioning, hydrophobic chromatography, and size and charge separation.[6]

*BCA is a patent of Pierce.

## References


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**Figure 1.**

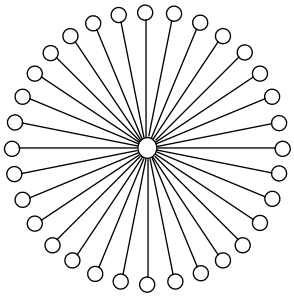


**Figure 2.**

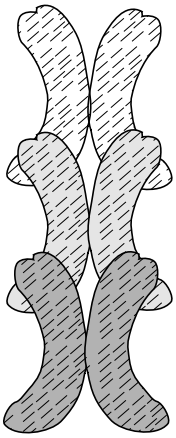
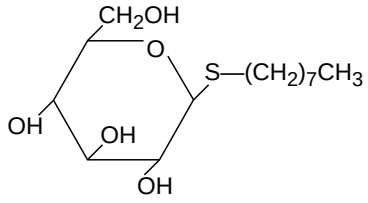


**Figure 3.**

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