Detergents

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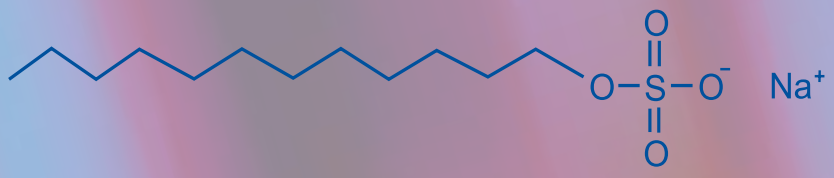

For Research and Diagnostics

- Non-Ionic Detergents
- Anionic Detergents
- Cationic Detergents
- Zwitterionic detergents
- Non-Detergent Sulfobetaine

Detergents

Detergents find a wide range of applications in biochemistry, cell and molecular biology, chemistry, diagnostics etc.

Typical applications are:

- Cell culture techniques
- Chemiluminescence and fluorescence analysis
- Chromatographic and electrophoretic separations
- Extraction of DNA and RNA
- Immunoassays
- Lysis of cells and tissues by membrane disintegration
- Permeabilization of cells
- Protein assays
- Reversed micellar extraction of peptides and proteins
- Solubilization of proteins
- Solubilization of photosystems and photosynthesis pigments
- Solubilization and characterization of phospholipids and lipid rafts
- Stabilization or reconstitution of proteins and protein complexes

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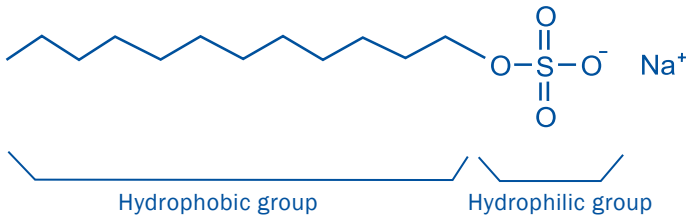
Broad range of detergents for your specific application(s)

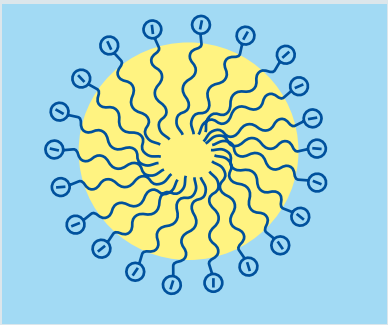
High quality detergents in convenient pack sizes at fair prices

Introduction

Detergents are a class of chemical substances which are characterized by their amphiphilic (amphipathic) structure. Each molecule contains at least one hydrophilic (polar) and one hydrophobic (lipophilic, non-polar) functional group.



The amphiphilic structure allows detergents to form highly organized spherical structures in aqueous solutions, the so-called micelles. The molecules aggregate with the hydrophilic groups directed to the outer side forming hydrogen bonds with the water molecules, while the non-polar groups remain inside the micelle due to hydrophobic interactions. This property is unique for detergents and the prerequisite for dissolving hydrophobic substances in water. Detergents are also surface active compounds, which reduce the surface tension of water (therefore also called surfactants): due to their amphipathic structure, they are adsorbed at interfaces, e.g. in aqueous solutions, the molecules are adsorbed at the water-air interface with the hydrophobic part in the air.



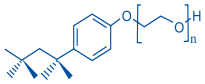
Most detergents are synthetic organic compounds (e.g. Tween® 80, sodium dodecyl sulfate). But there are also naturally occurring surfactants or derivatives of natural products (e.g. digitonin, sodium deoxycholate). According to their charge detergents can be divided into three classes:

- Ionic detergents
- Non-Ionic detergents
- Zwitterionic detergents

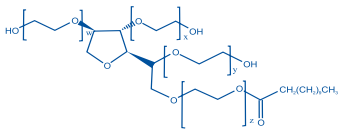
Non-ionic Detergents

They contain uncharged hydrophilic head groups that consist of:

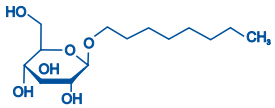
polyoxyethylene moieties
e.g. Tergitol™ 15-S-9



PEG-sorbitan units
e.g. Tween® 20



glycosidic groups
e.g. octyl-ß-D-glucopyranoside



They are well suited for breaking lipid-lipid and lipid-protein interactions but normally do not break protein-protein interactions. Compared to ionic detergents, they usually do not denature proteins and are therefore frequently used for the isolation of biologically active membrane proteins.

Non-ionic detergents

Description	Application	Size	Cat. no.
Brij 35™	▪ Isolation of functional membrane complexes ▪ Permeabilization of cells ▪ Preparation of yeast spheroplasts ▪ Protein extraction	100 g	15230.01
Digitonin	▪ Protein complex solubilization ▪ Permeabilization of certain cell types, e.g. blood platelets, hepatocytes, yeast and tumor cells	1 g	19550.02
Digitonin water soluble	▪ Protein complex solubilization ▪ Permeabilization of certain cell types, e.g. blood platelets, hepatocytes, yeast and tumor cells	250 mg	19551.01
		1 g	19551.02
Dodecyl-ß-D-maltoside	▪ Protein complex solubilization ▪ Investigation of photosynthetic membranes	1 g	20780.03
Polysorbate 80 VG	▪ For cell culture, enzymology, membrane research and other biochemical applications ▪ Vegetable origin	500 g	33116.01
		5 kg	33116.02
Tergitol™ 15-S-9	▪ Isolation, purification and analysis of membrane components ▪ Alternative for NP-40 and Triton X-100	100 ml	37242.01
		500 ml	37242.02
		2.5 L	37242.02
Tween® 20	▪ Suppression of unspecific reactions between antibodies, antigens and other molecules ▪ Solubilizer in membrane chemistry ▪ Density centrifugation of viruses	500 g	37470.01
		5 kg	37470.02
Tween® 80	▪ Cell culture suitable ▪ Solubilizitation of membrane proteins during isolation of membrane-protein complexes	500 g	37475.01
		5 kg	37475.02
Synperonic® F68		100 g	35724.01
		1 kg	35724.02
Synperonic® F108		100 g	35726.01
		1 kg	35726.02

Ionic Detergents

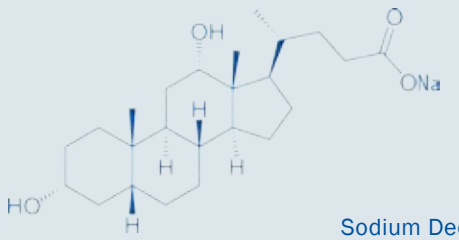
Because the head group is either negatively or positively charged, they can be sub-classified in anionic and cationic detergents.

Anionic detergents

An often-used example is the alkyl detergent sodium dodecyl sulfate (SDS). It carries a negatively charged sulfate group on a linear C12 hydrocarbon chain. SDS is considered as a very strong and biologically harsh surfactant. It can denature proteins by breaking intra- and intermolecular interactions and thus destroying their biological activity.

SDS

Other anionic detergents like bile acid salts have a rigid steroidal core structure. They do not carry a well-defined polar head group like SDS but the polar groups are distributed on different parts of the molecule. E.g. sodium deoxycholate carries a carboxylate group at the end of a short hydrocarbon chain and two hydroxyl groups on the steroid structure. The bile acids are less denaturing than the ionic alkyl detergents, possibly due to their rigid steroidal ring structure.



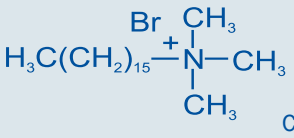
Sodium Deoxycholate

Anionic detergents

Description	Application	Size	Cat. no.
Cholic acid•Na-salt		500 g	17126.03
DOC (Deoxycholic acid•Na-salt)	▪ For bacteriology and enzymology ▪ Solubilization of many membrane proteins and phospholipids	25 g	18330.02
		100 g	18330.03
SDS (Dodecylsulfate•Na-salt)	▪ Protein solubilization ▪ SDS PAGE ▪ Reduction of non-specific binding sites on membranes during nucleic acid hybridization	250 g	20783.01
		1 kg	20783.02
SDS in Pellets (Dodecylsulfate•Na-salt)	▪ Pressed in small pellets thus avoiding the irritant dust of the powder form ▪ Protein solubilization ▪ SDS PAGE	100 g	20765.01
		250 g	20765.02
		1 kg	20765.03

Cationic detergents

The positively charged head group is often a quaternary ammonium group. Cetyltrimethylammoniumbromide (CTAB) carries a trimethylammonium group on a C16 hydrocarbon chain. It is a strong detergent which irreversibly denatures proteins.



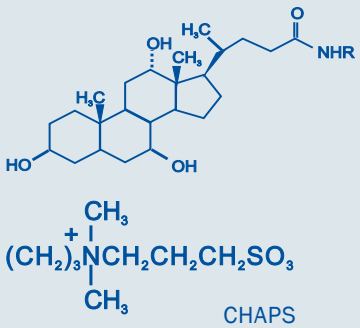
CTAB

Cationic detergents

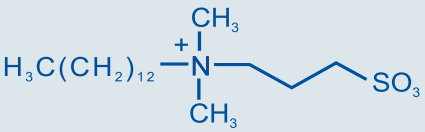
Description	Application	Size	Cat. no.
CTAB (Cetyltrimethyl ammonium•bromide)	▪ For acidic PAGE of highly positive charged and membrane proteins ▪ Solubilization of a wide variety of proteins and nucleic acids ▪ Cell permeabilization	100 g	16530.04
		500 g	16530.02

Zwitterionic Detergents

They carry a positively and a negatively charged group, but like non-ionic surfactants, do not have a net charge. Furthermore, they lack conductivity and electrophoretic mobility and do not bind to ion exchange resins. However, they are able - like ionic detergents - to break protein-protein interactions.



CHAPS



ASB-14

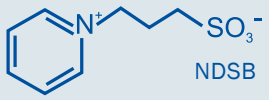
Zwitterionic detergents

Description	Application	Size	Cat. no.
CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate)	▪ Solubilizing and electrophoresis of membrane proteins ▪ Standard detergent for sample preparation and first dimension in 2D PAGE ▪ For enzyme immunoassay	1 g	17038.01
		5 g	17038.02
		25 g	17038.03
		100 g	17038.04
ASB-14 (3-[N,N-Dimethyl-(3-myristoylaminopropyl)-ammonio]-propanesulfonate)	▪ Solubilizing proteins for 2D PAGE ▪ Identification of previously undetected membrane proteins due to better protein solubilization properties than CHAPS	1 g	20757.01
		5 g	20757.02

Cationic detergents for irreversible denaturing of proteins

Non-Detergent Sulfobetaine (NDSB)

NDSB is a zwitterionic compound, but the hydrophobic chain is too short to form micelles. It improves the yield of membrane proteins when used with detergents and prevent aggregation of denatured proteins.



NDSB

Description	Application	Size	Cat. no.
NDSB-201 (3-(1-Pyridino)-1-propane sulfonate)	▪ A non-detergent sulfobetaine with zwitterionic properties, but does not form micelles ▪ Prevents protein aggregation ▪ Renaturation of chemically and thermally denatured proteins ▪ Solubilization of proteins for proteomic applications	250 g	20762.02

Non-detergent solubilisazion reagent

NDSB prevents aggregation of denatured proteins

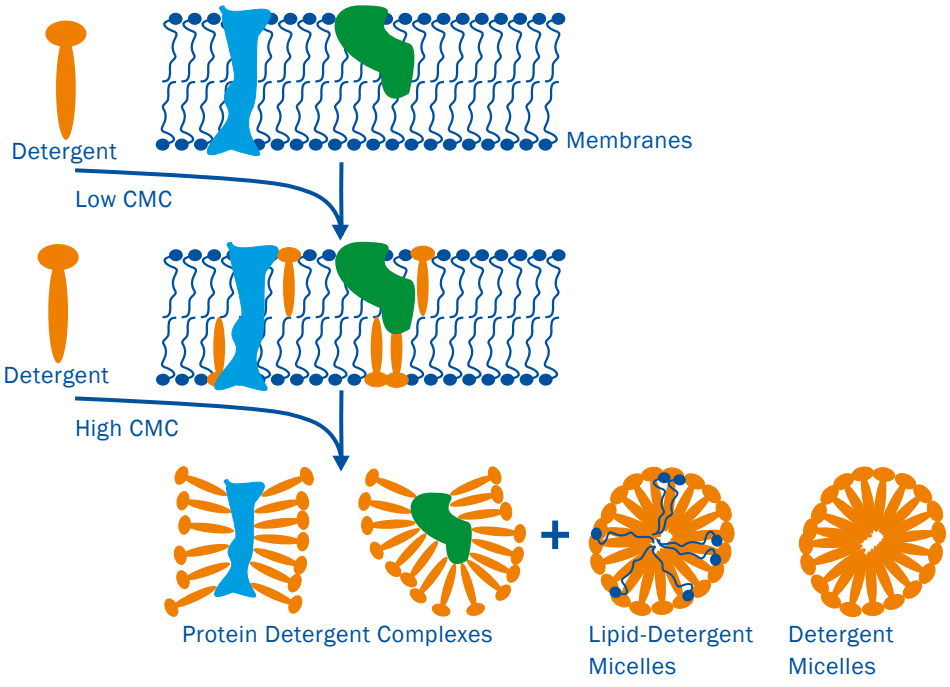
Physicochemical Properties of Detergents

Critical micellar concentration - CMC

The CMC is defined as the surfactant concentration at which formation of micelles begins. It is an important value, because it allows to determine the precise amount of detergent needed for the solubilization of proteins from lipid bilayers. Too little detergent results in inadequate solubilization and too much hinders detergent removal and can interfere with downstream processes.

The CMC value is characteristic for each surfactant. It is strongly influenced by environmental factors like pH, temperature and, for ionic detergents as well by ionic strength and type of counter ions. In solutions of ionic detergents, the increase in counter ion concentration reduces the CMC. The CMC is also influenced by the structure of the detergent, e.g. an increase in the length of the non-polar hydrocarbon chain will result in an increase of the micelle size and a lower CMC as fewer molecules are required to form a micelle. The CMC is also a measure for the hydrophobicity of a detergent and an indicator for the strength of detergent binding to proteins. Detergents with low CMC are generally more tightly bound than detergents with high CMC.

Schematic showing the stages of protein solubilisation with detergent



Aggregation number - Na

The aggregation number is the average number of molecules that form a micelle of the surfactant in question; it is hence a criterion for the micellar size.

The aggregation number is relevant for the solubilization of membrane components, but also for analytical separation procedures like dialysis or gel chromatography. Such as for the CMC, the Na is dependent on outer factors like pH, temperature and ionic strength.

Cloud point – cp

The cloud point is a typical property of polyethoxylated non-ionic surfactants. Above a specific temperature, the aqueous surfactant solution separates into a heavier, surfactant-rich and a lighter surfactant-depleted phase. The reason for this phase separation is the temperature-dependent dehydration of the polyoxyethylene chain. The temperature-induced phase separation can be utilized for biochemical separations, e.g. for the extraction of membrane proteins.

Hydrophilic-lipophilic balance – HLB

The HLB number is a measure of the hydrophilic or lipophilic character of surfactants. On a HLB-scale from 1 – 20, water/oil emulsifiers appear around 3 – 8, whilst hydrophilic surfactants (oil/water emulsifiers) range between 10 and 20. The HLB number can either be calculated from the chemical composition or it can be determined experimentally. HLB numbers for surfactant mixtures can be determined using a simple rule of mixtures.

Detergents - Overview

Detergent Class	MW	CMC* (mM)	Na*	HLB	Cloud Point (°C)
Non-ionic Detergents					
Brij 35®	ca. 1200	0.09	40	16.9	
Digitonin	1229.3	0.67 – 0.73	60	0.4	
Dodecyl-ß-D-maltoside (DDM)	510.6	0.15	98		
Synperonic F68	ca. 8300	0.04		29	
Synperonic F108	ca. 14000			27	
Tergitol™ 15-S-9	ca. 607	0.086	140	13.3	57.5 - 62.5 (1 % in H_2O)
Tween® 20	ca. 1200	0.059	-	16.7	76 (3 % in 1 N NaCl solution)
Tween® 80	ca. 1300	0.01	-	15.0	65 (3 % in 1 N NaCl solution)
Ionic Detergents					
Cetyltrimethyl ammonium·bromide (CTAB)	364.5	0.92	61	-	
Cholic acid·Na-salt	430.5	7 – 16.2	2 - 7	18	
Deoxycholic acid·Na-salt (DOC)	414.6	2.4 - 5	2 – 19.9	16	
Dodecylsulfate·Na-salt (SDS)	288.4	8.1	60 - 62	40	
Zwitterionic Detergents					
ASB-14	434.7	8	-	-	
CHAPS	614.9	4.2 – 6.5	9 – 10		
Non-Detergent Sulfobetaine					
NDSB 201	201.4	No micelles are formed			

*refer to 20 – 25 °C

Selecting a Detergent

Finding the right detergent for a special application, can be a difficult and tedious task.

Here some hints how to select:

- conduct a survey of scientific publications on this topic
- start with a detergent which has been used successfully for the isolation of a similar membrane protein, enzyme or receptor
- try other detergents with similar or slightly different properties
- if the isolation process involves a dialysis step, detergents with a high CMC are preferred as they bind less strongly to proteins than detergents with low CMC
- for electrophoretic separation or ion exchange chromatography, non-ionic or zwitterionic detergents are recommended

To maintain the biological activity of an isolated membrane protein, it may be necessary to test not only different detergents, but also each detergent under different conditions, e.g. concentration, pH, buffer composition etc. Generally, non-ionic and zwitterionic detergents are milder than ionic ones and better suited to preserve biological and enzymatic activity.

Detergents for native and 2D PAGE

In native sample preparation, you need to add non-ionic detergents to improve the solubility of hydrophobic and membrane proteins. They do not interfere with the electrophoretic run, but result in less streaking and better resolution.

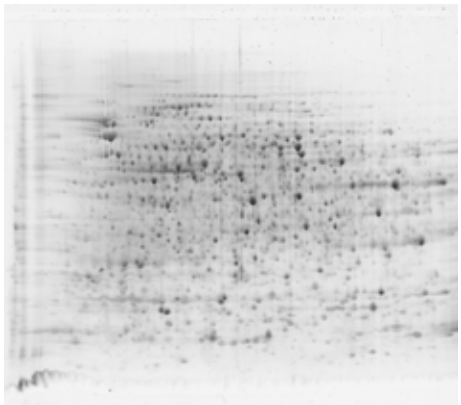
Highly recommended for Blue Native PAGE are digitonin and dodecyl-beta-D-maltoside. With digitonin even intact protein super complexes can be isolated.

Due to the large diversity of proteins present in different cells and tissues it is necessary to optimize the detergent concentration. Dodecyl-beta-D-maltoside is used in a final concentration of 0.5 % - 5 % and digitonin of 0.5 % - 2.5 %.

Prior to 2D gel electrophoresis, non-ionic or zwitterionic detergents are added to disrupt hydrophobic interactions and increase solubility of proteins at their pI.

The zwitterionic detergent CHAPS as a 4 % lysis solution is preferable to non-ionic detergents such Tergitol™ 15-S-9 because of higher solubilization efficiency, especially for integral membrane proteins.

Furthermore, Tergitol is not compatible with downstream applications such as mass spectrometry. Some hydrophobic membrane proteins could only be solubilized with novel zwitterionic detergents like ASB-14.

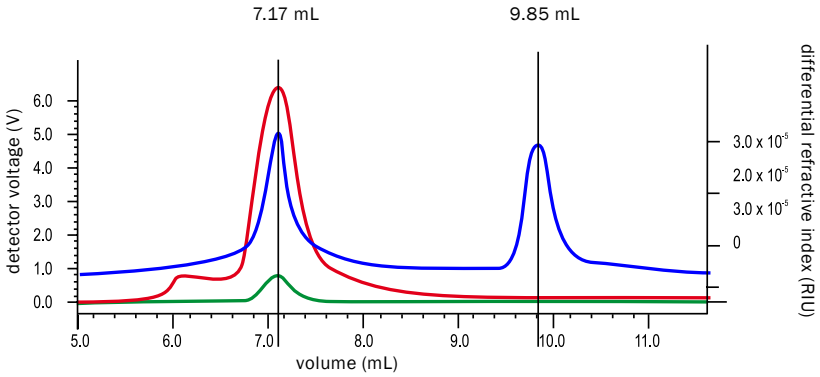


Detergent Removal

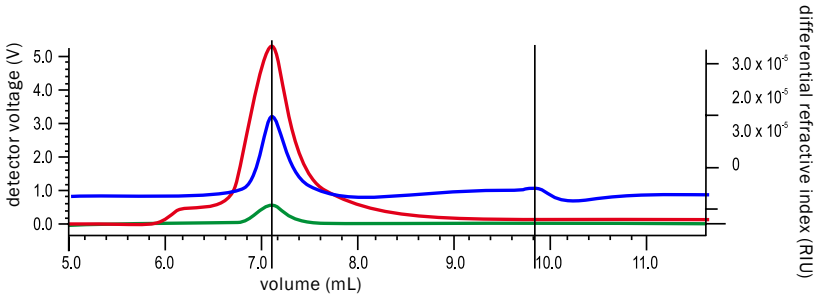
Proteus Detergent Anion Exchange Mini Spin Columns are designed for rapid and effective removal of free detergents micelles and complete detergent exchange. They are optimized for membrane proteins with pI <8 in complex with non-ionic or zwitterionic detergents. Simple and adaptable to your protein requiring only a microfuge for operation.

Ideal for applications such as ELISA, IEF, MS and NMR which suffer from interference with excess detergents.

- Universal appeal as most proteins have a pI between 4 - 8
- Rapid removal and exchange of free detergent micelles in 10 min
- Generate concentrated proteins free of detergent micelles
- Only requires a microfuge for use



SAMPLE 1: Dimeric Photosystem II (PSII) membrane protein concentrated 273 times. Note the free detergent micelles eluting at 9.85 ml.



SAMPLE 2: SAMPLE 1 after elution from the Proteus Detergent Anion Exchange Mini Spin Column with 75 mM MgSO4 in detergent-depleted buffer. Note the effectiveness of the Proteus spin columns to eliminate free detergent micelles.

Product	Size	Cat. no.
Proteus Detergent Anion Exchange Mini Spin Column Kit	4 columns	42240.01
Proteus Detergent Anion Exchange Mini Spin Column Trial Kit	20 columns	42241.01

Remove detergents and concentrate your protein in only 10 min

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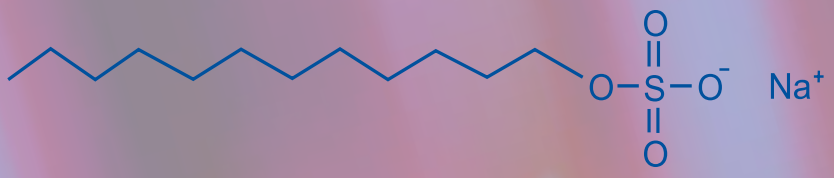

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