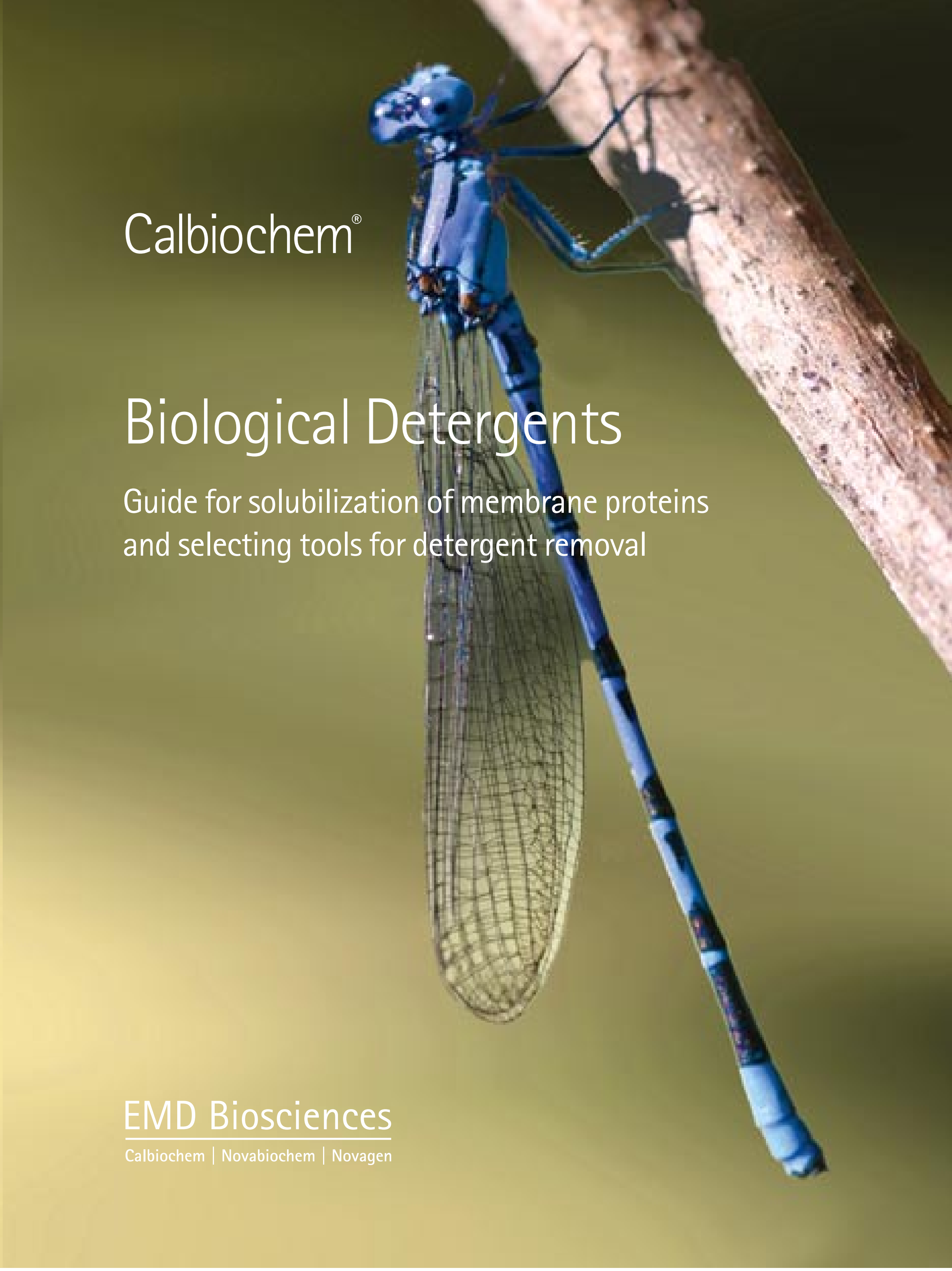


Calbiochem®

Biological Detergents

Guide for solubilization of membrane proteins and selecting tools for detergent removal

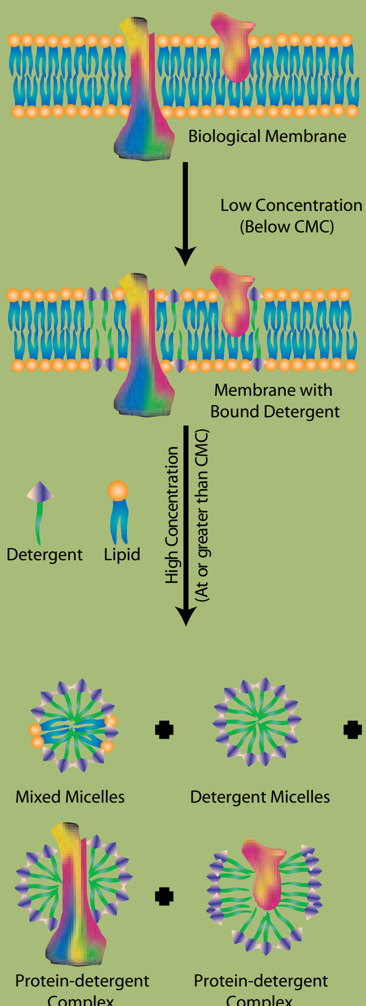
EMD Biosciences

Calbiochem | Novabiochem | Novagen

EMD

Biological Properties and Uses of Detergents

Solubilization of Cell Membranes with Detergents



Biological membranes, composed of complex assemblies of lipids and proteins, serve as physical barriers in the cell and are sites for many cellular signaling events. The majority of membrane lipids contain two hydrophobic hydrocarbon tails connected to a polar head group. This architecture allows lipids to form bilayer structures in which the polar head groups are exposed outwards towards the aqueous environment and the hydrophobic tails are sandwiched between the hydrophilic head groups. Integral membrane proteins are held in the membrane by hydrophobic interactions between the hydrocarbon chains of the lipids and the hydrophobic domains of the proteins.

In order to understand the function and structure of membrane proteins, it is necessary to carefully isolate these proteins in their native form in a highly purified state. It is estimated that about one third of all membrane-associated proteins are integral membrane proteins, but their solubilization and purification is more challenging because most of these proteins are present at very low concentrations. Although membrane protein solubilization can be accomplished by using amphiphilic detergents, preservation of their biological and functional activities can be a challenging process as many membrane proteins are susceptible to denaturation during the isolation process. Detergents solubilize membrane proteins by mimicking the lipid bilayer environment. Micelles formed by the aggregation of detergent molecules are analogous to the bilayer of the biological membranes. Proteins can incorporate into these micelles by hydrophobic interactions. Hydrophobic regions of membrane protein, normally embedded in the membrane lipid bilayer, are surrounded by a layer of detergent molecules and the hydrophilic portions are exposed to the aqueous medium. This property allows hydrophobic membrane proteins to stay in solution.

Detergents are amphipathic in nature and contain a polar group at one end and long hydrophobic carbon chain at the other end. The polar group forms hydrogen bonds with water molecules, while the hydrocarbon chains aggregate via hydrophobic interactions. At low concentrations, detergent molecules exist as monomers. When the detergent monomer concentration is increased above a critical concentration, detergent molecules self associate to form thermodynamically stable, non-covalent aggregates known as micelles. The critical micelle concentration (CMC) is an important parameter for selecting an appropriate detergent. At the CMC, detergents begin to accumulate in the membrane. The effective CMC of a detergent can also be affected by other components of the biological system, such as lipids, proteins, pH, ionic strength, and temperature of the medium. An important point to note here is that any addition of salts to ionic detergents, such as SDS, may reduce their CMC because salt would tend to reduce the repulsion between the charged head groups. Here micelles will form at a lower concentration.

At low concentrations, detergents merely bind to the membrane by partitioning into the lipid bilayer. As the concentration of detergent increases, the membrane bilayer is disrupted and is lysed, producing lipid-protein-detergent mixed micelles. Any further increase in detergent concentration will produce a heterogeneous complex of detergent, lipid-detergent, and protein-detergent mixed micelles. In the detergent-protein mixed micelles, hydrophobic regions of the membrane proteins are surrounded by the hydrophobic chains of micelle-forming lipids.

Excessive amounts of detergents are normally used for solubilization of membrane proteins to ensure complete dissolution and provide for a large number of micelles to give one micelle per protein molecule. For further physiochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent. Excess amounts of detergents can be removed by hydrophobic absorption on a resin, gel chromatography (based on the difference in size between protein-detergent, lipid-detergent, and homogenous detergent micelles), ion-exchange chromatography (based on the charge difference between protein-detergent and protein-free detergent micelles), or by dialysis. Detergents with high CMC can be readily removed from protein-detergent complexes by dialysis, whereas low CMC detergents dialyze away very slowly.

Guidelines For Selecting a Detergent

A membrane protein is considered solubilized if it is present in the supernatant after one hour centrifugation of a lysate or a homogenate at 100,000 x g. In most cases, the biological activity of the protein be preserved in the supernatant after detergent solubilization. Hence, the appropriate detergent should yield the maximum amount of biologically active protein in the supernatant. Given the large number of detergents available today, choosing an appropriate detergent can be a difficult process. Some of the points outlined below can be helpful in selecting a suitable detergent.

- First survey of the literature. Try a detergent that has been used previously for the isolation and characterization of a protein with similar biochemical or enzymological properties should be tried first.

- Consider the solubility of the detergent at working temperature. For example, ZWITTERGENT® 3-14 is insoluble in water at 4°C while TRITON® X-114 undergoes a phase separation at room temperature.

- Consider the method of detergent removal. If dialysis is to be employed, a detergent with a high CMC is clearly preferred. Alternatively, if ion exchange chromatography is utilized, a non-ionic detergent or a ZWITTERGENT® is the detergent of choice.

- Preservation of biological or enzymological activity may require experimenting with several detergents. Not only the type but also the quantity of the detergent used will affect the protein activity. For some proteins biological activity is preserved over a very narrow range of detergent concentration. Below this range the protein is not solubilized and above a particular concentration, the protein is inactivated.

- Consider downstream applications. Since TRITON® X-100 contains aromatic rings that absorb at 260-280 nm, this detergent should be avoided if the protocols require UV monitoring of protein concentration. Similarly, ionic detergents should be avoided if the proteins are to be separated by isoelectric focusing. For gel filtration of proteins, detergents with smaller aggregation numbers should be considered.

- Consider detergent purity. Detergents of utmost purity should be used since some detergents such as TRITON® X-100 are generally known to contain peroxides as contaminants. The Calbiochem® PROTEIN GRADE® or ULTROL® GRADE detergents that have been purified to minimize these oxidizing contaminants are recommended.

- Calbiochem also offers a variety of Molecular Biology Grade detergents for any research where contaminants such as DNase, RNase, and proteases are problematic.

- A non-toxic detergent should be preferred over a toxic one. For example, digitonin, a cardiac glycoside, should be handled with special care.

- For as yet unknown reasons, specific detergents often work better for particular isolation procedures. For example, EMPIGEN® BB (Cat. No. 324690) has been found to be the most efficient detergent for the solubilization of keratins while preserving their antigenicity. Similarly, *n*-Dodecyl-β-D-maltoside (Cat. No. 324355) has been found to be the detergent of choice for the isolation of cytochrome c oxidase. Hence, some "trial and error" may be required for determining optimal conditions for isolation of a membrane protein in its biologically active form.

- In some cases, it has been observed that the inclusion of non-detergent sulfobetaines (NDSBs) with detergents in the isolation buffer dramatically improves yields of solubilized membrane proteins.

See page 9.

Technical Support
Phone 800 628 8470
E-mail calbiochem@emdbiosciences.com

Types of Detergents: Main Features

Type of Detergent	Examples	Comments
Ionic Detergents	Anionic: Sodium dodecyl sulfate (SDS) Cationic: Cetyl methyl ammonium bromide (CTAB)	• Contain head group with a net charge. • Either anionic (- charged) or cationic (+ charged). • Micelle size is determined by the combined effect of hydrophobic attraction of the side chain and the repulsive force of the ionic head group. • Neutralizing the charge on the head group with increasing counter ions can increase micellar size. • Useful for dissociating protein-protein interactions. • The CMC of an ionic detergent is reduced by increasing the ionic strength of the medium, but is relatively unaffected by changes in temperature.
Non-ionic Detergents	TRITON®-X-100, *n*-octyl-β-D-glucopyranoside	• Uncharged hydrophilic head group. • Better suited for breaking lipid-lipid and lipid-protein interactions. • Considered to be non-denaturants. • Salts have minimal effect on micellar size. • Solubilize membrane proteins in a gentler manner, allowing the solubilized proteins to retain native subunit structure, enzymatic activity and/or non-enzymatic function. • The CMC of a non-ionic detergent is relatively unaffected by increasing ionic strength, but increases substantially with rising temperature.
Zwitterionic Detergents	CHAPS, ZWITTERGENTS	• Offer combined properties of ionic and non-ionic detergents. • Lack conductivity and electrophoretic mobility. • Do not bind to ion-exchange resins. • Suited for breaking protein-protein interactions.

Non-detergent Sulfobetaines

Product	Cat. No.	M. W.	Size
NDSB-195	480001	195.3	5 g 25 g
NDSB-201	480005	201.2	25 g 250 g
NDSB-211	480013	211.3	1 g 5 g
NDSB-221	480014	221.3	5 g 25 g
NDSB-256	480010	257.4	5 g 25 g
NDSB-256-4T	480011	257.4	5 g 25 g
NDSB Set	480012		1 set

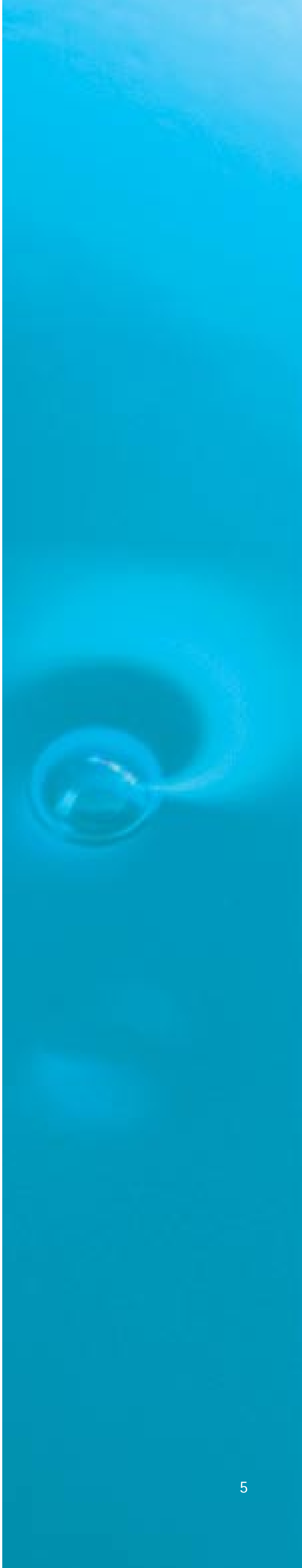
Ionic Detergents

Product	Cat. No.	M. W.* (anhydrous)	CMC‡ (mM)	Size
Cetyltrimethylammonium Bromide, Molecular Biology Grade	219374	364.5	1.0	100 g
Chenodeoxycholic Acid, Free Acid	2204	392.6		5 g
Chenodeoxycholic Acid, Sodium Salt	220411	414.6		5 g
Cholic Acid, Sodium Salt	229101	430.6	9-15	50 g 250 g
Cholic Acid, Sodium Salt, ULTROL® Grade	229102	430.6	9-15	1 g 5 g
Deoxycholic Acid, Sodium Salt	264101	414.6	4-8	25 g 100 g 1 kg
Deoxycholic Acid, Sodium Salt, ULTROL® Grade	264103	414.6	2-6	5 g 25 g 100 g
Glycocholic Acid, Sodium Salt	360512	487.6	7.1	1 g 5 g
Glycodeoxycholic Acid, Sodium Salt	361311	471.6	2.1	5 g
Glycolithocholic Acid, Sodium Salt	36217	455.6		100 mg
Glycoursodeoxycholic Acid, Sodium Salt	362549	471.6		1 g
Lauroylsarcosine, Sodium Salt	428010	293.4		5 g
LPD-12	437600	2839.4	< 0.001	1 mg 2 mg
Sodium *n*-Dodecyl Sulfate	428015	288.4	7-10	1 kg
Sodium *n*-Dodecyl Sulfate, High Purity	428016	288.5	7-10	25 g
Sodium *n*-Dodecyl Sulfate, Molecular Biology Grade	428023	288.4	7-10	50 g 500 g
Sodium *n*-Dodecyl Sulfate, 20% Solution (w/v)	428018	288.4	7-10	200 ml
Taurochenodeoxycholic Acid, Sodium Salt	580211	521.7		1 g 5 g
Taurocholic Acid, Sodium Salt	580217	537.7	3-11	5 g 25 g
Taurocholic Acid, Sodium Salt, ULTROL® Grade	580218	537.7	3-11	1 g 5 g
Taurodeoxycholic Acid, Sodium Salt	580221	521.7	1-4	5 g 50 g
Tauroursodeoxycholic Acid, Sodium Salt	580549	521.7		1 g 5 g
Ursodeoxycholic Acid, Sodium Salt	672305	414.6		1 g

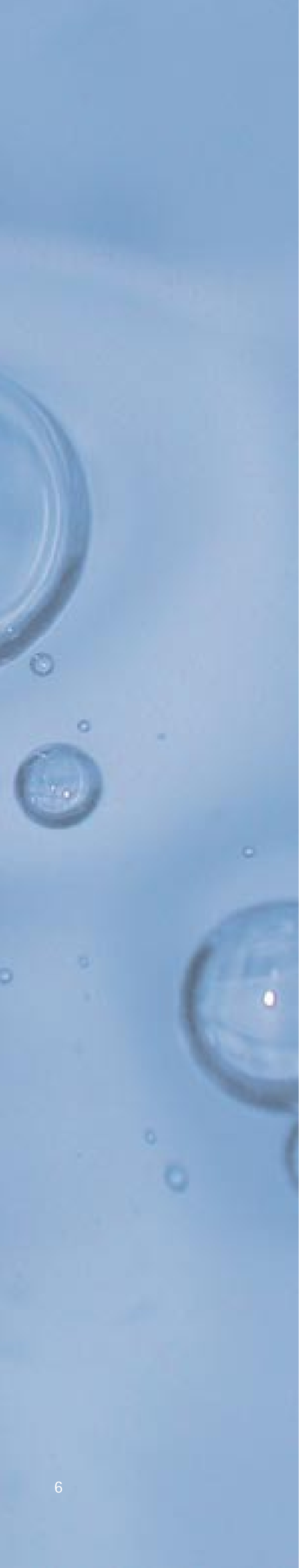
Key:

* : Average molecular weights are given for detergents composed of mixtures of different chain lengths.

‡ : Temperature = 20-25°C.



Technical Support
Phone 800 628 8470
E-mail calbiochem@emdbiosciences.com



Non-ionic Detergents

Product	Cat. No.	M. W.* (anhydrous)	CMC‡ (mM)	Size
APO-10	178375	218.3	4.6	1 g
APO-12	178377	246.4	0.568	1 g
Big CHAP	200965	878.1	3.4	1 g
Big CHAP, Deoxy	256455	862.1	1.1-1.4	250 mg 1 g
BRIJ® 35 Detergent, 30% Aqueous Solution	203724	1199.6	0.092	100 ml 1 l
BRIJ® 35 Detergent, PROTEIN GRADE®, 10% Solution, Sterile-Filtered	203728	1199.6	0.092	50 ml
$C_{12}E_8$	205528	538.8	0.110	1 g
$C_{12}E_8$, PROTEIN GRADE® Detergent, 10% Solution	205532	538.8	0.110	1 set
$C_{12}E_9$, PROTEIN GRADE® Detergent, 10% Solution	205534	582.8	0.080	1 set
Cyclohexyl-*n*-hexyl-β-D-maltoside, ULTROL® Grade	239775	508.6	0.560	1 g
n-Decanoylsucrose	252721	496.6	2.5	1 g 5 g
n-Decyl-β-D-maltopyranoside, ULTROL® Grade	252718	482.6	1.6	1 g 5 g
Digitonin, Alcohol-Soluble, High Purity	300411	1229.3		250 mg 1 g
Digitonin, High Purity	300410	1229.3		250 mg 1 g 5 g
n-Dodecanoylsucrose	324374	524.6	0.3	1 g 5 g
n-Dodecyl-β-D-glucopyranoside	324351	348.5	0.130	1 g
n-Dodecyl-β-D-maltoside, ULTROL® Grade	324355	510.6	0.1-0.6	500 mg 1 g 5 g 25 g
ELUGENT™ Detergent, 50% Solution	324707			100 ml
GENAPOL® C-100, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	345794	627		50 ml
GENAPOL® X-080, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	345796	553	0.06-0.15	50 ml
GENAPOL® X-100, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	345798	641	0.15	50 ml
HECAMEG	373272	335.4	19.5	5 g
n-Heptyl-β-D-glucopyranoside	375655	278.3	79	1 g
n-Heptyl-β-D-thioglucopyranoside, ULTROL® Grade, 10% Solution	375659	294.4	30 (remains unchanged between 1 and 20°C)	10 ml 50 ml
n-Hexyl-β-D-glucopyranoside	376965	264.3	250	1 g

Orders Phone 800 854 3417
Fax 800 776 0999
Web www.emdbiosciences.com/calbiochem

Non-ionic Detergents *continued*

Product	Cat. No.	M. W* (anhydrous)	CMC‡ (mM)	Size
MEGA-8, ULTROL® Grade	444926	321.5	58	1 g 5 g
MEGA-9, ULTROL® Grade	444930	335.5	19-25	5 g
MEGA-10, ULTROL® Grade	444934	349.5	6-7	5 g
n-Nonyl-β-D-glucopyranoside	488285	306.4	6.5	1 g
NP-40 Alternative	492016		0.05-0.3	100 ml 500 ml 1000 ml
NP-40 Alternative, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	492018		0.05-0.3	50 ml 500 ml
n-Octanoylsucrose	494466	468.5	24.4	5 g
n-Octyl-β-D-glucopyranoside	494459	292.4	20-25	500 mg 1 g 5 g 25 g
n-Octyl-β-D-glucopyranoside, ULTROL® Grade	494460	292.4	20-25	250 mg 1 g 5 g
n-Octyl-β-D-maltopyranoside	494465	454.5	23.4	1 g
n-Octyl-β-D-thioglucopyranoside, ULTROL® Grade	494461	308.4	9	5 g
PLURONIC® F-127, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	540025	12,500 (avg.)	4-11	50 ml
Saponin	558255			100 g
TRITON® X-100 Detergent	648462	650 (avg.)	0.2-0.9	1 kg 3 kg
TRITON® X-100, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	648463	650 (avg.)	0.2-0.9	50 ml
TRITON® X-100 Detergent, Molecular Biology Grade	648466	650 (avg.)	0.2-0.9	50 ml
TRITON® X-100 Detergent, Hydrogenated	648465	631 (avg.)	0.25	10 g
TRITON® X-100, Hydrogenated, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	648464	631 (avg.)	0.25	10 ml
TRITON® X-114, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	648468	537 (avg.)	0.35	50 ml
TWEEN® 20 Detergent	655205	1228 (avg.)	0.059	250 ml
TWEEN® 20 Detergent, Molecular Biology Grade	655204	1228 (avg.)	0.059	100 ml
TWEEN® 20, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	655206	1228 (avg.)	0.059	50 ml
TWEEN® 80, PROTEIN GRADE® Detergent, 10% Solution, Sterile-Filtered	655207	1310 (avg.)	0.012	50 ml

Key:

* : Average molecular weights are given for detergents composed of mixtures of different chain lengths.

‡ : Temperature = 20-25°C.

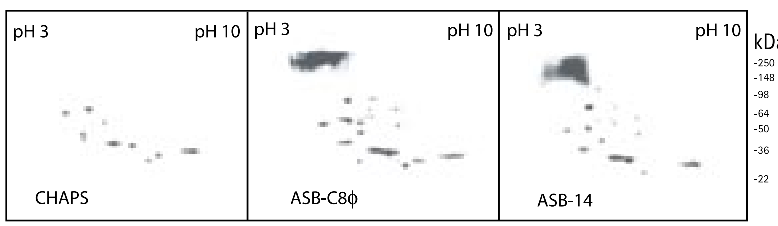
Technical Support
Phone 800 628 8470
E-mail calbiochem@emdbiosciences.com

Zwitterionic Detergents

The recent growing interest in analysis and identification of the total protein complement of a genome (proteomics) has prompted efforts in improving the existing techniques in two-dimesional gel electrophoresis (2-DGE). The invention of immobilized pH gradients (IPGs) (1) to eliminate cathodic drift and the introduction of thiourea (2) as a powerful chaotrope are two such examples. However, solubilization of hydrophobic, notably membrane-type, proteins remains a great challenge in 2-DGE.

CHAPS is a sulfobetaine-type zwitterionic detergent, which has been employed in combination with nonionic detergents (e.g. TRITON® X-100, NP-40) in 2-DGE to minimize the loss of membrane proteins due to hydrophobic interactions between the proteins (which can cause problems in protein extraction), and between the proteins and the IPG matrix (which can cause problems in the recovery of proteins in 2-DGE). Chevallet et al. (3) have identified several new sulfobetaine-type zwitterionic detergents, among them are ASB-14, ASB-16, and ASB-C8Ø, which show improved efficiency in protein solubilization with 2-DGE.

Similar to CHAPS, these newly discovered detergents contain a polarized sulfobetaine head group, followed by a three-carbon linkage between the quaternary ammonium and the amido nitrogen. What makes them different from CHAPS is that they contain mainly linear hydrocarbon tails that are composed of 13 to 16 carbons. This allows for higher urea compatibility and, in some instances, improved membrane protein recovery in 2-DGE as compared to CHAPS. Henningsen et al. (4) have shown that ASB-C8Ø was better than CHAPS at solubilizing an ion channel and a G-protein-coupled receptor. Using red blood cell ghosts as a model, Tastet et al. (5) have shown that detergents such as ASB-14, ASB-16 and ASB-C8Ø provide greater protein solubilization efficiency and, in general, better pattern and quality in 2-DGE than detergents with carboxybetaine hydrophilic heads or longer hydrophobic tails.



References:

1. Bjellqvist, B., et al. 1982. *J. Biochem. Biophys. Methods* **6**, 317.
2. Rabilloud, T., et al. 1997. *Electrophoresis* **18**, 307.
3. Chevallet, M., et al. 1998. *Electrophoresis* **19**, 1901.
4. Henningsen, R., et al. 2002. *Proteomics* **2**, 1479.
5. Tastet, C., et al. 2003. *Proteomics* **3**, 111.

Zwitterionic Detergents

Product	Cat. No.	M. W.	CMC‡ (mM)	Size
ASB-C7BzO	182729	399.6		1 g 5 g
ASB-14	182750	434.7		5 g 25 g
ASB-14-4	182751	448.7		1 g 5 g
ASB-16	182755	462.7		5 g 25 g
ASB-C6Ø	182728	412.6		1 g 5 g
ASB-C8Ø	182730	440.6		1 g 5 g
CHAPS	220201	614.9	6-10	1 g 5 g 10 g 25 g

Zwitterionic Detergents *continued*

Product	Cat. No.	M. W.	CMC‡ (mM)	Size
CHAPSO	220202	630.9	8	1 g 5 g
DDMAB	252000	299.5	4.3	5 g
DDMAU	252005	397.7	0.13	5 g
EMPIGEN® BB Detergent, 30% Solution	324690	272	1.6-2.1	100 ml
PMAL-B-100	528200	9000		1 g
ZWITTERGENT® 3-08 Detergent	693019	279.6	330	5 g
ZWITTERGENT® 3-10 Detergent	693021	307.6	25-40	5 g 25 g 100 g
ZWITTERGENT® 3-12 Detergent	693015	335.6	2-4	5 g 25 g
ZWITTERGENT® 3-14 Detergent	693017	363.6	0.1-0.4	5 g 25 g 100 g
ZWITTERGENT® 3-16 Detergent	693023	391.6	0.01-0.06	5 g 25 g

‡ : Temperature = 20-25°C.

Detergent Sets

Product	Cat. No.	Description	Size
APO Detergent Set	178400	Contains 1 g each of the following non-ionic detergents: APO-8, APO-9, APO-10 (Cat. No. 178375), APO-11, and APO-12 (Cat. No. 178377).	1 set
ASB ZWITTERGENT® Set	182753	Contains 1 g each of the following zwitterionic amidosulfobetaine (ASB) detergents: ASB-14 (Cat. No. 182750), ASB-16 (Cat. No. 182755), and ASB-C8ϕ (Cat. No. 182730)	1 set
Detergent Test Kit	263451	Contains 1 g each of the following detergents: *n*-Hexyl-β-D- glucopyranoside (Cat. No. 376965), *n*-Heptyl-β-D-glucopyranoside (Cat. No. 375655), *n*-Octyl-β-D-glucopyranoside, ULTROL® Grade (Cat. No. 494460), *n*-Nonyl-β-D-glucopyranoside (Cat. No. 488285), and *n*-Dodecyl-β-D-maltopyranoside, ULTROL® Grade (Cat. No. 324355).	1 kit
Detergent Variety Pack	263458	Contains 1 g each of the following components: CHAPS (Cat. No. 220201), Deoxycholic Acid, Sodium Salt, ULTROL® Grade (Cat. No. 264103), *n*-Octyl-β-D-glucopyranoside (Cat. No. 494459), *n*-Octyl-β-D-thioglucopyranoside ULTROL® Grade (Cat. No. 494461), and ZWITTERGENT® 3-14 (Cat. No. 693017).	1 pack
NDSB Set	480012	Contains 5 g each of NDSB-195 (Cat. No. 480001), NDSB-256 (Cat. No. 480010), and 25 g of NDSB-201 (Cat. No. 480005).	1 set
ProteoExtract® Detergent Set	539751	Contains the following detergents: 10 g of TRITON® X-100 (Cat. No. 648462) and 1 g each of ASB-14 (Cat. No. 182750), ASB 14-4 (Cat. No. 182751), ASB-16 (Cat. No. 182755), C8ϕ (Cat. No. 182730), CHAPS (Cat. No. 220201), *n*-Dodecyl-β–D-maltopyranoside, ULTROL® Grade (Cat. No. 324355), and ZWITTERGENT® 3-10 (SB 3-10, Cat. No. 693021).	1 set
ZWITTERGENT® Test Kit	693030	Contains 1 g each of the following components: ZWITTERGENT® 3-08 (Cat. No. 693019), ZWITTERGENT® 3-10 (Cat. No. 693021), ZWITTERGENT® 3-12 (Cat. No. 693015), ZWITTERGENT® 3-14 (Cat. No. 693017), and ZWITTERGENT® 3-16 (Cat. No. 693023).	1 kit

Technical Support
Phone 800 628 8470
E-mail calbiochem@emdbiosciences.com

Removal of Unbound Detergents

Excess detergent is normally employed in solubilization of membrane proteins. This helps to ensure complete dissolution of the membrane and to provide a large number of micelles such that only one protein molecule is present per micelle. However, for further physicochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent. Several methods have been used for detergent removal that take advantage of the general properties of detergents: hydrophobicity, CMC, aggregation number, and the charge. Four commonly used detergent removal methods follow:

Hydrophobic Adsorption

This method exploits the ability of detergents to bind to hydrophobic resins. For example, CALBIOSORB™ Adsorbent is a hydrophobic, insoluble resin that can be used in batchwise applications to remove excess detergent. Generally, a solution containing a detergent is mixed with a specific amount of the resin and the mixture is allowed to stand at 4°C or room temperature. The resin with the bound detergent can be removed by centrifugation or filtration. This technique is effective for removal of most detergents. If the adsorption of the protein to the resin is of concern, the resin can be included in a dialysis buffer and the protein dialyzed. However, this usually requires extended dialyzing periods.

Size Exclusion Chromatography

Gel chromatography takes advantage of the difference in size between protein-detergent, detergent-lipid, and homogeneous detergent micelles. In most situations protein-detergent micelles elute in the void volume. The elution buffer should contain a detergent below its CMC value to prevent protein aggregation and precipitation. Separation by gel chromatography is based on size. Hence, parameters that influence micellar size (ionic strength, pH, and temperature) should be kept constant from experiment to experiment to obtain reproducible results.

Dialysis

When detergent solutions are diluted below the CMC, the micelles are dispersed into monomers. The size of the monomers is usually an order of magnitude smaller than that of the micelles and thus can be easily removed by dialysis. If a large dilution is not practical, micelles can be dispersed by other techniques such as the addition of bile acid salts. For detergents with high CMC, dialysis is usually the preferred choice.

Ion exchange Chromatography

This method exploits the differences in charge between protein-detergent micelles and protein-free detergent micelles. When non-ionic or zwitterionic detergents are used, conditions can be chosen so that the protein-containing micelles are adsorbed on the ion-exchange resin and the protein-free micelles pass through. Adsorbed protein is washed with detergent-free buffer and is eluted by changing either the ionic strength or the pH. Alternatively, the protein can be eluted with an ionic detergent thus replacing the non-ionic detergent.

Detergent Removal Products

Product	Cat. No.	Description	Size
CALBIOSORB™ Adsorbent	206550	Off-white beads slurried in 100 mM sodium phosphate buffer, 0.1% NaN_3, pH 7.0. Designed for the removal of detergents from protein solutions and other biological mixtures in aqueous medium.	50 ml
CALBIOSORB™ Adsorbent, Prepacked Columns	206552	Designed for the removal of detergents from protein solutions and other biological mixtures in aqueous medium. Each set contains three columns. Each column has a 10 ml total volume (5 ml resin bed in 100 mM sodium phosphate, 0.1% NaN_3, pH 7.0 with a 5 ml buffer reservoir) and an upper frit to protect the resin bed from disruption.	1 set
Detergent-OUT™, Detergent Removal Kit	263455	A simple and rapid column-based method to remove detergents such as TRITON® X-100 Detergent, NP-40, CTAB, CHAPS, Lubrol, TWEEN® Detergent, sodium deoxycholate, and others from protein solutions. Simply load protein solutions onto column and spin. Detergent is retained by the column matrix and the protein is collected in a small volume. Offered as a mini kit to process samples containing up to 3 mg detergent, or as a medi kit to process samples containing up to 15 mg detergent.	1 mini 1 medi
Detergent-OUT™, SDS Removal Kit	263454	A simple and rapid column based method to remove free SDS from protein solutions. Simply load protein solutions onto column and spin. The detergent is retained by the column matrix and the protein is collected in a small volume. An SDS test kit is provided for determining detergent removal efficiency. Offered as a mini kit with the capacity to remove 2 mg of SDS from solution or as a medi kit with the capacity to remove up to 10 mg of SDS from the protein solution.	1 mini 1 medi

CALBIOSORB™ Adsorbent

Solubilization of membranes by detergents is essential for their characterization and reconstitution. However, subsequent removal of detergents, particularly the non-ionic detergents with low CMC values, is difficult to achieve. Dialysis, the most common method of detergent removal, usually requires about 200-fold excess of detergent-free buffer with three to four changes over several days. However, it is ineffective for removal of detergents with low CMC values. In addition, prolonged exposure to detergents during dialysis can damage certain membrane proteins. Gel filtration, another common method for detergent removal, is highly effective in the reconstitution of AChR, (Ca^{2+} + Mg^{2+})-ATPase, and lactose transporters. However, it gives a broader size distribution of vesicles compared to the dialysis method. Therefore, an expeditious alternative in reconstitutional studies is the prior removal of detergents by using a resin capable of effectively binding nondialyzable detergents of low CMC. We offer an excellent detergent removal product, CALBIOSORB Adsorbent. CALBIOSORB is a hydrophobic resin that is processed to eliminate unbound organic contaminants, salts, and heavy metal ions and is especially formulated for detergent removal from aqueous media. It is supplied in 100 mM sodium phoshate buffer pH 7.0, containing 0.1% sodium azide and can be easily re-equilibrated with any other buffer prior to use.

The following table highlights the adsorptive capacity of CALBIOSORB Adsorbent as tested for a variety of commonly used detergents.

Detergent Adsorption Capacity of CALBIOSORB Adsorbent

Detergent	Cat. No.	M.W.	Detergent Type	Adsorption Capacity (mg detergent/ml resin)
Cetyltrimethylammonium Bromide (CTAB)	219374	364.5	Cationic	120
CHAPS	220201	614.9	Zwitterionic	110
Cholic Acid, Sodium Salt	229101	430.6	Anionic	73
n-Dodecyl-β-D-maltoside, ULTROL® Grade	324355	510.6	Non-ionic	66
n-Hexyl-β-D-glucopyranoside	376965	264.3	Non-ionic	78
n-Octyl-β-D-glucopyranoside, ULTROL® Grade	494460	292.4	Non-ionic	132
Sodium Dodecyl Sulfate (SDS)	428015	288.5	Anionic	94
TRITON X-100, PROTEIN GRADE® Detergent	648463	650 (avg.)	Non-ionic	157
TWEEN 20, PROTEIN GRADE® Detergent	655206	1228.0 (avg.)	Non-ionic	122

Note: Detergent adsorption capacities were measured by allowing 1.0 g of buffer-free CALBIOSORB™ Adsorbent to equilibrate at room temperature with an excess of detergent (10 ml of 2.0% detergent in H_2O) for 24 hours, then measuring the amount of unadsorbed detergent remaining in the supernatant by gravimetric analysis.

For more information or to order Calbiochem products, contact EMD Biosciences

Orders

Phone 800 854 3417

Fax 800 776 0999

Technical Support

Phone 800 628 8470

E-mail calbiochem@emdbiosciences.com

Visit our website

www.emdbiosciences.com/calbiochem

You can also order Calbiochem products through our distribution partner, VWR International

Phone 800 932 5000

Web www.vwr.com

Printed in the USA

EMD Biosciences

Calbiochem | Novabiochem | Novagen

P.O. Box 12087
La Jolla, CA 92039-2087

CHANGE SERVICE REQUESTED

PRSRT STD
U.S. POSTAGE
PAID
MILWAUKEE, WI
PERMIT NO. 4078

CB0733-2006USD
Detergents