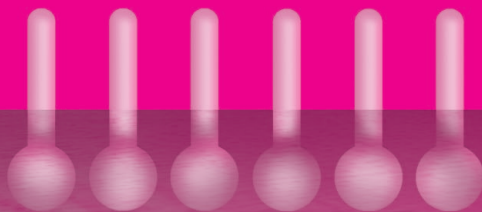


# Detergents



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## Detergents – more than foam!

Detergents are widely used in biochemistry, cell biology or molecular biology. Cell lysis, protein solubilization, protein crystallization or reduction of background staining in blotting experiments are just a few of numerous applications. The goal of this brochure is to summarize the important facts from the literature, which may help the reader to select a detergent for his special application. Detergents can be classified for instance according to their chemical structure stating their constituent polar and nonpolar group (glucosides, alkyl ionic detergents, polyoxyethylene alcohols, bile salts, sulphonates etc.), the charge character (anionic, cationic, zwitterionic = amphoteric and nonionic) or simply whether they are mild or strong in terms of their ability to solubilize and / or to denature proteins. They all have in common that they are soluble amphipathic (amphiphilic) compounds, with both lipophilic (hydrophobic, nonpolar) and lipophilic (hydrophilic, polar) sites within one molecule. Therefore, they will form stable aggregates (micelles) above a critical concentration, called **CMC value** (Critical Micellar Concentration). This value is specific to each detergent and different factors may influence it:

**The temperature:** The equilibrium monomer concentration increases with temperature and reaches the CMC level at the critical micellar temperature (CMT) which is thus the lowest temperature at which micelles can form (Helenius & Simons 1975). Most detergents have a CMT below 0°C and therefore do not precipitate in the cold as does SDS. At the CMT, cloudy crystalline suspensions of a detergent suddenly become clear. Especially the cloud point is to be considered with the polyethylene glycol polar group - detergents (such as Triton® X-100). At the cloud point, these detergents separate as a pure phase from the aqueous solution, resulting in a dramatically increased micellar weight. The cloud point effect is caused by a decrease in head group hydration. The cloud point of Triton® X-100 in water is 64°C, but is lowered by higher ionic strength and by additives. Further, the Krafft point is the temperature above which the solubility of a detergent rises sharply. At this temperature the solubility of the detergent becomes equal to the CMC (according to IUPAC).

**The chemical structure:** The CMC decreases as the hydrophobic character of the lipophilic site increases. Correspondingly, the polar head group of ionic detergents will antagonize to micelle formation resulting in detergents with very high CMC values. In other words, ionic detergents generally have higher CMCs than nonionic detergents. Nonionic detergent micelles are, due to their frequently high aggregation numbers, approximately of the same size as macromolecules and thus subject to the limitations in diffusion and migration rates characteristics of macromolecules in the molecular weight range of 10,000 to 1,000,000.

**The salt concentration** (neutral salts; ionic strength): The CMC decreases with increasing salt concentrations and ionic strength, while the micellar size increases. Zwitterionic and nonionic detergents are less sensitive, while ionic detergents such as SDS or bile salts show strong effects. The zwitterionic detergents have permanent bound counterions and nonionic detergents are uncharged and are therefore less sensitive to changes in the salt concentration or ionic strength, respectively. When performing ion-exchange chromatography and isoelectric focusing, it is important to consider the ionic strength, since the starting conditions frequently vary from the final conditions. The pH value is of importance for these techniques too.

**The pH value:** If a detergent possesses a titratable polar group, a direct effect on changes in the pH is to be expected. A very dramatic effect is seen with cholate and deoxycholate, which become protonated below pH 7.8, and the micelle size then increases dramatically. Lauroylsarcosinate as well contains a carboxylic acid function which will be protonated at relatively weak acidic pH values. The technique of isoelectric focusing may span the pH range from 3 to 11.

Above the CMC, free monomer molecules are in equilibrium with the micelles and the solubilizing ability increases. In aqueous solutions, the hydrophilic site will be on the outside of the micelle, and the lipophilic portion inside. In organic solvents, reversed micelles form, with the lipophilic site outside. Detergents with high CMC values are more easily removed by dialysis.

The critical micellar concentration is frequently used for selecting a detergent. This value is determined in aqueous solutions in the presence of the detergent of interest and water (e.g. Chattopadhyay & London 1984). Therefore, these assay conditions will not reflect the situation in complex biological fluids or lysates. In other words, influences of other compounds in these mixtures are not included. If non-standardized methods are applied to determine CMC values (e. g. presence of salts or buffers), variations will be inevitable. Nevertheless, the CMC value is informative and useful for making a selection.

Other parameters that describe the detergent behavior are the aggregation number (N) and the Hydrophile-Lipophile Balance number (HLB). The aggregation number N is the average number of monomers per micelle, which depends on the molecular mass of the detergent and factors like the ionic strength. It tends to be low for small polar molecules like the bile salts and much higher for bulky detergents like Triton®. Aggregation number may rise sharply with ionic strength, e.g., from 2.2 to 22 for deoxycholate and from 2.8 to 4.8 for cholate, when salt concentration is increased from 10 mM to 150 mM



(Paradies 1980, Furth *et al.* 1984). HLB is an index of the hydrophilicity of a detergent and probably less suited to be used as selection criterion (Tanford & Reynolds 1976). The larger the HLB, the more hydrophilic the detergent.

When selecting a detergent for investigating integral membrane proteins a first consideration is to first solubilize the membranous structure and second to stabilize the protein once extracted. Integral membrane proteins are likely to need the presence of detergent in all subsequent procedures, to mimic the lipids of their natural environment. As a rule-of-thumb, 1 mg of membrane requires a minimum of 2 mg of detergent. This is approximately 1 mg of detergent for each mg of protein and 1 mg for each mg of lipid. (Helenius *et al.* 1979; Scopes 1994).

## Criteria for selecting a detergent

Choosing the best detergent for a special application is not trivial. In many cases, a set of detergents has to be tested to select the one with the best properties. If the goal of the isolation of a protein is to preserve its structural and functional state, one should consider the temperature, the pH and the ionic strength of the system or interference with assays. Several other parameters may influence the choice too. Assuming that the factors mentioned above are dictated by the protein and are held constant, the factors are left to be optimised are detergent head group, detergent tail group, and detergent concentration (Neugebauer 1990, Tanford & Reynolds 1976).

**Nonionic polyoxyethylene detergents** (e. g. Brij<sup>®</sup>, Nonidet<sup>®</sup>, Triton<sup>®</sup>, Tween<sup>®</sup>) do not usually denature proteins. They are suited to investigate the subunit structure of membrane proteins, since they won't break the interactions. Polyoxyethylene detergents are subject to autooxidation. Traces of heavy metal ions can act as catalysts. Butylated hydroxytoluene (1:500 molar ratio to detergent) is usually sufficient to prevent autooxidation (Helenius *et al.* 1979). Alternatively, the solutions of the detergents can be treated with a mixed-bed resin to remove oxidised products (Schubert *et al.* 1983). AppliChem offers peroxide-free ready-to-use solutions of all these polyoxyethylene detergents. Another drawback of this class of detergents is their absorption in the UV range of light, due to the presence of the phenyl ring (Tanford & Reynolds 1976). Some divalent cations (e. g. Fe<sup>2+</sup>, Ca<sup>2+</sup>, Co<sup>2+</sup>) can cause precipitation of these detergents (Helenius *et al.* 1979).

**Bile salts** (e.g. cholate, deoxycholate) are based on the rigid steroid ring structure. They belong to the group of ionic detergents, but don't possess a polar head group like other detergents. Instead the polar groups are distributed in different parts of the molecule, all on one side of the molecule. They effectively solubilize membranes. Please note that these bile salts precipitate, when the pH value of the buffer is one unit above the pK<sub>a</sub> value of the bile salts (pK<sub>a</sub> deoxycholate 6.2; pK<sub>a</sub> cholate 5.2). Divalent cations (e. g. Mg<sup>2+</sup>, Ca<sup>2+</sup>) precipitate both detergents (Helenius *et al.* 1979).

**Alkyl ionic detergents** (e. g. SDS, CTAB, DTAB) are nearly always denaturants at concentrations and temperatures that have to be employed to solubilize membranes. They usually separate subunits of complex proteins. The functioning of ionic detergents strongly depends on the ionic strength and on the nature of the counterion. Increasing counterion concentrations will lower the CMC and increase the micellar size. Potassium salts of alkyl sulfates or salts with divalent cations are insoluble at room temperature (Helenius *et al.* 1979).

**Solubility:** Many detergents have limited water-solubility at elevated temperatures. On warming a solution, separation into an aqueous phase containing little detergent, and a heavier, detergent-rich phase will occur (phase separation; Scopes 1994, Bordier 1981). Some detergents are not readily water-soluble at room temperature (e. g. CTAB) or +4°C and at this low temperature, most proteins / cell extracts have to be handled. SDS precipitates at temperatures below 10 - 15°C. In the presence of potassium ions, precipitation is enhanced. This feature may be applied for removing SDS from samples (Suzuki & Terada 1988)

**Denaturing or inactivation of proteins:** If the native biological structure and activity of a protein has to be preserved, mild detergents have to be applied. Octylglucoside, CHAPS, Triton<sup>®</sup> X-100, Digitonin and sodium cholate have been proven to be a good choice for membrane proteins. Most of the strong (effective) detergents, such as SDS or CTAB, will denature the protein, often irreversibly.

**Removal from samples:** When detergents have to be removed from a sample, dialysis is the method of choice. Octylglucoside, the MEGA-series of detergents, CHAPS, sodium cholate besides several other detergents fulfil this criteria. Again, detergents with high CMC values are more easily removed by dialysis.

**Interference with protein activities:** The chemical nature should be considered e.g. when used in enzyme activity assays, protein determination etc. They should not serve as an enzyme substrate. Glucoside detergents can be cleaved by glucosidases.

**Absorption in the UV region:** Absorption of the detergent in the UV region (280 nm) will interfere with protein determination. Interference with colorimetric assays is unwanted too. Octylglucoside doesn't absorb at 280 nm.

**Electrical charge and applicability in ion-exchange chromatography:** Charged detergents are less used in chromatography. Usually, nonionic and zwitterionic detergents have to be chosen. Both groups of detergents behave essentially as uncharged molecules. They do not move in electrical fields, do not bind to ion-exchange resins, and do not contribute to the net charge of macromolecules to which they are bound (Hijlmeland 1990).

**Efficiency of extracting the protein:** In most cases, the detergent will be selected according to its solubilization capability of the desired protein. Several considerations will influence the choice, such as keeping the detergent concentration as low as possible. Keep in mind that not all detergents will separate subunits of proteins. This may lead to wrong estimations of the molecular weight.

**CMC value and micelle size:** Detergents are quite small molecules, but form micelles which may be of similar size to proteins. The micelle size increases and the CMC decreases with increasing size of the lipophilic part of the detergent and, to a lesser extent, with decreasing size and polarity of the polar groups (Helenius *et al.* 1979). Detergents with a high CMC form smaller micelles. Unfortunately, they have the disadvantage of being less efficient as detergents.

selecting

# Isolation of Membrane Proteins

(summarized from Hjelmeland & Chrambach 1984, Hjelmeland 1990a)

The isolation of membrane proteins seems to be more complicated than the resolution and reconstitution of soluble proteins. The association of a membrane protein with the lipid bilayer and sometimes with other proteins makes the solubilization more difficult. Once isolated, the protein requires a more complex environment in terms of maintenance of structure and functionality. Each individual protein has to be treated in an individual manner. This section will summarize a few important parameters outlined primarily by Hjelmeland and Chrambach (1984) that have to be considered when unknown proteins are to be isolated.

I. The first consideration concerns the **solubility** of the protein. It has to be distinguished between soluble and insoluble proteins. To test for this, a suitable assay for the activity has to be established. This can be an assay determining the enzymatic activity, the binding behaviour etc. In case the protein requires a ligand/co-factor for its activity, it has to be controlled whether such a ligand has been separated during isolation of the protein.

II. The **choice** of a **suitable** detergent is the second step.

- a.) If the detergent has to be removed, it is recommended to select a detergent with a high CMC value and a low molecular weight. This type of surfactant is usually easy to be removed by dialysis.
- b.) If the absorbance at 280 nm is an important parameter, Triton® X-100 must be excluded.
- c.) If the separation technique is based on charge differences (e.g. ion exchange chromatography or electrophoresis), charged detergents should be avoided.
- d.) If divalent cations are essential for function, those that form insoluble complexes (e.g. all bile salts, such as sodium cholate with its carboxylic acid group, Lauroylsarcosinate) should be omitted.
- e.) If precise physical data are to be obtained, detergents with known physical parameters must be used.

III. Other parameters of importance are the choice of the **buffer** system, the **temperature** and the **ionic strength**.

- a.) The buffer contributes a stable pH environment. Concentrations of 25 to 50 mM are normally sufficient. Phosphate buffers have good solubilizing properties. Depending on the system, concentrations between 0.1 and 0.5 M are applied.
- b.) For effects of the temperatures see above.
- c.) The ionic strength is a consideration, since the proteins do have ionic and hydrophobic interactions. Therefore, sodium chloride at concentrations between 150 mM and 500 mM is frequently included.
- d.) Chaotropic salts and urea are part of many solubilization buffers. Urea forms complexes with all nonionic detergents. Many detergents, such as the nonionics and the linear alkyl sulfobetaines, are insoluble in moderate concentrations of guanidine hydrochloride (Hjelmeland 1990a).

IV. The **initial solubilization** assay will provide information on the success of maintaining the functionality during the isolation of the protein. In case that the activity is low or even absent, stabilizer can be added (e.g. protease inhibitors, glycerol, DTT)

V. Once a detergent has been selected, the optimum ratio of protein-to-detergent has to be determined for optimum solubilization. The initial experiments won't give this information. The solubilization is based on the dispersion of the phospholipids by detergent molecules, forming mixed micelles, and finally by their replacement (solubilized complexes). At low detergent concentrations, detergent molecules will bind to the membrane. Increasing the detergent concentration to one tenth of the protein concentration will lead to lysis of the membrane. Solubilization occurs at a detergent to protein ratio of 1 – 2 and delipidation at 10 to 20 (Helenius & Simons 1975). Since these data are generated with less complex viral systems, this model may well be an oversimplification. The optimal ratio is determined experimentally at several detergent concentrations for three different protein concentrations. The recommended starting concentrations of the protein are 5 mg/ml, 7.5 mg/ml and 10 mg/ml, respectively. The detergent is applied at multiples of 1.5 and 2 of 5 mg/ml. Lower protein concentrations are not useful, since detergents with a high CMC may not be present at solubilizing concentrations for a given detergent-to-protein ratio. Solubilization occurs at or near the CMC for most detergents. Hence, low protein concentrations may lead to slightly higher values for detergent-to-protein ratios when detergents with high CMC values are used. A protocol for initial solubilization experiments is provided by Hjelmeland (1990a) with very useful explanations.

A different but attractive method has been applied by Bordier (1981). This approach makes use of the phase separation of the nonionic detergent Triton® X-114 at temperatures above 20°C, separating into an aqueous and a detergent phase. While the hydrophilic proteins will stay in the aqueous phase, the amphiphilic proteins will be enriched in the detergent phase. The technique has been improved with time (Pryde 1986).

# Reconstitution of Membrane Proteins

(summarized from Klausner *et al.* 1984, Racker 1979)

Integral membrane proteins behave as amphiphiles: they possess hydrophilic and hydrophobic domains. In aqueous solutions, the hydrophobic surfaces of the protein favor to self-associate, leading to the formation of insoluble aggregates in most cases (van Renswoude & Kempf 1984). Therefore, as mentioned before, they require a more complex environment to show full activity after isolation. Reconstitution describes the process of reincorporation of a solubilized membrane protein into a natural or artificial membrane. The phospholipids of choice are usually phosphatidylethanolamine, -serine, -inositol and -choline. Only the correct orientation and insertion into a lipid bilayer will allow functional reconstitution of activity. Several approaches for the reconstitution are described in detail in both references, e.g.:

- **Detergent-Dialysis Procedure:** Removal of the detergent cholate by a mixture of phospholipids during dialysis. Advantage: simple method; Disadvantage: Long exposure to the detergent; time factor (20 hr – 4 d);
- **Detergent-Dilution Procedure:** Dilution of the detergent with a reconstitution mixture containing about 0.5 – 0.8 % cholate allowed assay to be performed; Advantage: rapid; Disadvantage: cannot replace Dialysis Procedure in all systems;
- **Sonication Procedure:** Originally for detergent-free proteins that are mixed with phospholipids and sonicated. Advantages: rapid, no detergent required; Disadvantage: power output of sonicator difficult to reproduce. New method with a freeze-thaw step: Liposomes are sonicated first, then addition of protein and quickly frozen; again sonication; Advantage: reduced sonication time;
- **Incorporation Procedure:** Preformed liposomes are incubated with dilute solutions of membrane proteins in the presence of small amounts of detergent;
- **Fusion Procedure:** Particular value for the reconstitution of mixtures of proteins that are optimally reconstituted by different procedures; makes use of the fusion of liposomes by the addition of  $\text{Ca}^{2+}$ ; additional advantage: results in large liposomes;

While Racker focusses on the reconstitution of membrane enzymes, Klausner describes several examples of reconstitution of membrane receptors. See Rivnay and Metzger (1982) for the reconstitution of an immunoglobulin receptor into liposomes.

## Removal of Detergents from Proteins

(summarized from Furth *et al.* 1984, Hjelmeland 1990b)

The removal of detergents in the course of the purification of a protein may be advisable at several stages of the purification procedure. The initial solubilization of a protein is usually achieved with an excess of detergent. This excess may interfere with subsequent chromatographic steps. Another step in the purification protocol, where a detergent has to be removed, can be the exchange of the detergent by another one with better properties for further purification. At least for the (final) analytical assays, the removal of the detergent is necessary in most cases. Examples: Ionic detergents interfere with ion-exchange chromatography or isoelectric focusing.

Exchange procedures can be chromatographic techniques, dialysis, or precipitation of the protein, e.g. by polyethylene glycol (Ingham 1990). As mentioned before, detergents with a high CMC value are more easily removed, because they bind less tightly to the proteins as compared to detergents with a low CMC value (e.g. nonionic detergents). Another parameter affecting the ease of detergent removal is the micellar molecular weight (MMW). Detergents with a large MMW are difficult to remove by dialysis or ultrafiltration (e.g. most nonionic detergents incl. Triton® X-100), but in the case of Triton® X-100 easily removed by an ion-exchange resin (Cheetham 1979). Micelles of these detergents may contain more detergent molecules than protein, thus dominating the properties of the micelle. For more details and typical protocols see Hjelmeland (1990b) and Furth *et al.* (1984).

i s o l a



# Solubilization of Protein Aggregates

(from Marston & Hartley 1990)

Today, many proteins are expressed in *E. coli* by genetic engineering. This, or any other expression system, is capable of producing high levels of proteins expressed from cloned genes. In most cases, the proteins expressed at high levels in *E. coli* will aggregate in an insoluble form, the so called inclusion bodies. Principally, aggregation is advantageous, since the inclusion bodies can easily be recovered, leaving the problem of solubilizing and refolding the aggregated proteins. Once isolated, the inclusion bodies have to be washed to remove unwanted proteins associated with or entrapped in the inclusion bodies. Reagents that release eukaryotic polypeptides from inclusion bodies into solution are guanidine hydrochloride (5 - 8 M), urea (6 - 8 M), SDS, alkaline pH (<9.0) or organic solvents, such as acetonitrile/propanol (see Table I from Marston & Hartley 1990). Other buffers may include lysozyme, EDTA, DNase and a detergent like deoxycholate or Nonidet®. For further details of the refolding and purification of proteins from aggregates, I refer you to Marston & Hartley 1990.

## “Single-Case Studies”

### SDS – The Noble Detergent

The anionic detergent sodium dodecyl sulfate (SDS) is maybe the most popular detergent used in biological research, worth to dedicate a separate section. It disrupts non-covalent bonds in the proteins, thereby denaturing them, causing the molecules to lose their native conformation. If a protein consists of several polypeptide chains, they become dissociated. Monomeric SDS binds tightly to most proteins at about 1.4 mg SDS/mg protein (Nielsen & Reynolds 1978; see Table I this ref.), corresponding to a ratio of one SDS anion for every two amino acid residues. This confers a negative charge on the protein that is proportional to the mass of that protein. The electrostatic repulsion that is created by binding of SDS causes proteins to unfold into a rod-like shape thereby eliminating differences in shape as a factor for separation in the gel. Combined treatment with a disulfide reducing agent ( $\beta$ -mercaptoethanol or dithiothreitol) completely unfolds the protein. Polyacrylamide gel electrophoresis in the presence of SDS separates the polypeptide chains according to their molecular weight. Thus the molecular weight of the polypeptide chains of a given protein can be determined by comparing their electrophoretic mobility on SDS gels to the mobility of marker proteins with polypeptide chains of known molecular weights. This most popular electrophoretic method is the SDS-PAGE system developed by Laemmli (1970). There are a few restrictions, since proteins with a molecular weight below 15,000 or glycoproteins are particularly prone to quite anomalous interaction with SDS (Tanford & Reynolds 1976).

**Binding of SDS to water-soluble proteins:** At room temperature, some proteins, including pepsin, papain, and glucose oxidase (if unreduced) do not bind SDS in a cooperative fashion with accompanying unfolding even if the SDS concentration is increased to the CMC (or above). Many viral protein capsids (stabilized mainly by protein-protein interactions) share this resistance towards SDS. The massive cooperative mode of binding can be induced for all these proteins by heating in the presence of SDS. (RNase A, for instance, is denatured by SDS but not by DTAB at 25°C). Most membrane proteins are denatured by SDS, and it is usually assumed that these proteins show a cooperative mode of binding identical to that of water-soluble proteins. This is indeed the basis of the use of SDS gel electrophoresis to estimate the molecular weights of reduced polypeptide chains.

**Removal of SDS from protein solution:** When proteins have been isolated in the presence of SDS, in many cases the detergent has to be removed for investigation of the protein (e. g. sequence analysis). Dialysis, acetone precipitation of the protein, anion exchange resins or precipitation with barium, all are disadvantageous in terms of loss of protein or insufficient removal of SDS. Potassium salts effectively precipitate SDS, leaving proteins in the supernatant. Experiments from Suzuki & Terada (1988) have shown that quantities as low as 0.05 % SDS to 1 % could be removed by potassium phosphate (20 mM potassium salt are enough for 0.5 % = 17.3 mM SDS).

## Triton® X-100 – Attractive, but Beware!

This polyoxyethylene-derived nonionic detergent with an alkylphenyl hydrophobic group is one of the popular detergents as well, but differs very much from the ionic detergent SDS. It is relatively nondenaturing. Another favourable and outstanding property is its suitability for the solubilization of membranes for the isolation of many integral membrane proteins. Triton® X-100 inhibits protein-protein and protein-lipid bonding. It has a bulky polar head group and a small CMC value. Therefore, the micellar size (MMW) is large, causing problems when this detergent has to be removed from solutions by dialysis. Fortunately, other methods for removal are available, like adsorption to the ion-exchange resin Amberlite XAD-2 (Cheetham 1979). Phase separation is another keyword relating to Triton® X-100. At temperatures above the cloud point of 64°C, the detergent separates into a detergent phase and aqueous phase.

Impurities of several nonionic detergents (Brij® series, Triton® series, Tween® series) led to preparation artifacts of proteins. During storage, light and oxygen probably cause the formation of peroxides of these detergents (Chang & Bock 1980). The authors measured the concentration of sulfhydryl oxidizing agents in a 1 % solution of Triton® X-100, rising from less than 1 µM in a fresh solution to 136 µM within 8 weeks in closed bottles at room temperature. The peroxides on their part will oxidize sulfhydryl groups in proteins, leading to e.g. formation of dimers (Schubert *et al.* 1983). AppliChem offers products of all three series as 10 % peroxide-free solutions, purified with mixed bed ion-exchange resins. Last but not least, Triton® X-100 interferes with the Folin - Lowry protein assay.

Lipid rafts are domains based on clustered sphingolipids and cholesterol that have been proposed to exist in the plasma membrane, differing from the normal membrane composition in terms of (phospho)lipids and proteins. They are resistant to nonionic detergents at low temperatures (+4°C), such as Triton® X-100. When Triton® X-100 is added to cells, the fluid membrane will be solubilized while the lipid rafts will remain intact and can be extracted. Lipid rafts float to the top of a sucrose gradient. The term lipid rafts has been introduced in 1988 by research groups in Germany (K. Simons) and The Netherlands (G. van Meer). The original function and even the existence of lipid rafts is still a very controversial issue (for ref. see e.g. (and ref. therein) Schuck *et al.* 2003; Lai 2004; Rajendran & Simons 2005, Pike 2004, Heerklotz 2002, Mongrand 2004).

## At a glance

### Interference with protein assays

according to Stoscheck 1990 (modified)

Substance	Lowry*	BCA*	Bradford*	colloidal gold*	UV* 280 nm	UV* 205 nm
Brij® 35		1 %	X		1 %	1 %
CHAPS	1 mM	1 %	1 %		10 %	<0.1 %
Deoxycholate	0.0625 %		0.25 %		0.3 %	0.1 %
Digitonin					10 %	
Nonidet® P-40		1 %	X		X	
Octylglucoside		1 %	2 %		10 %	
SDS	1.25 %	1 %	0.1 %	0.1 %	0.1 %	0.1 %
Triton® X-100	0.25 %	1 %	0.1 %	1 %	0.02 %	<0.01 %
Tween® 20	0.1 %	1 %	X	1 %	0.3 %	0.1 %

X = interferes; \* tolerable limit

### Detergents by techniques

according to Neugebauer 1990 (modified)

Technique	Detergents
Blotting, hybridization	SDS
Cell lysis (nucleic acid preparation)	SDS, CTAB
Electrophoresis, Electrofocusing	SDS, Nonidet® P-40
Enzyme immunoassays	Tween® 20, Triton® X-100
Liposome preparation	Cholate, Deoxycholate, Octylglucoside
Protein crystallization	Octylglucoside, Maltosides
Selective solubilization of membranes	Triton® X-100

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## Detergents (Tensides)

Prod.-No.	Description	M	Formula	CMC (25°C)	HLB	Class
A6834	Benzethonium chloride	448.08	C <sub>27</sub> H <sub>42</sub> ClNO <sub>2</sub>			cationic
A1381	Brij® 35	1198.56	C <sub>58</sub> H <sub>118</sub> O <sub>24</sub>	0.09 mM	16.9	nonionic
A1669	Brij® 58	1123.51	C <sub>56</sub> H <sub>114</sub> O <sub>21</sub>	0.077 mM	15.7	nonionic
A0742	Cetylpyridinium chloride monohydrate	358.01	C <sub>21</sub> H <sub>38</sub> ClN · H <sub>2</sub> O	0.12 mM		cationic
A0805/A6284	Cetyltrimethylammonium bromide	364.46	C <sub>19</sub> H <sub>42</sub> BrN	0.92 mM		cationic
A1099/A4011	CHAPS	614.89	C <sub>32</sub> H <sub>58</sub> N <sub>2</sub> O <sub>5</sub> S	6.5 mM		zwitterionic
A1100	CHAPSO	630.87	C <sub>32</sub> H <sub>58</sub> N <sub>2</sub> O <sub>6</sub> S	8 mM		zwitterionic
A1018	1-Decanesulfonic acid sodium salt	244.33	C <sub>10</sub> H <sub>21</sub> NaO <sub>3</sub> S			anionic
A3208	n-Decyl-β-D-glucopyranoside	320.43	C <sub>16</sub> H <sub>32</sub> O <sub>6</sub>	2.2 mM (20°C)		nonionic
A6816/A6769	n-Decyl-β-D-maltoside	482.57	C <sub>22</sub> H <sub>42</sub> O <sub>11</sub>	1.8 mM		nonionic
A1698	Deoxy-BIGCHAP	862.07	C <sub>42</sub> H <sub>73</sub> N <sub>3</sub> O <sub>16</sub>	1.1 - 1.4 mM		nonionic
A1905	Digitonin	1229.34	C <sub>56</sub> H <sub>92</sub> O <sub>29</sub>			nonionic
A1019	1-Dodecanesulfonic acid sodium salt	272.38	C <sub>12</sub> H <sub>25</sub> NaO <sub>3</sub> S	9.8 mM	12.3	anionic
A5890	n-Dodecyl-β-D-glucopyranoside	348.48	C <sub>18</sub> H <sub>36</sub> O <sub>6</sub>	0.19 mM		nonionic
A0819/A6817	Dodecyl-β-D-maltoside	510.63	C <sub>24</sub> H <sub>46</sub> O <sub>11</sub>	0.15 - 0.19 mM		nonionic
A4147	Dodecyltrimethylammonium bromide	308.35	C <sub>15</sub> H <sub>31</sub> BrN	15 mM		cationic
A1384	HECAMEG	335.39	C <sub>15</sub> H <sub>29</sub> NO <sub>7</sub>	19.5 mM		nonionic
A3708	1-Heptanesulfonic acid sodium salt anhydrous	202.25	C <sub>7</sub> H <sub>15</sub> NaO <sub>3</sub> S			anionic
A1015	1-Heptanesulfonic acid sodium salt monohydrate	220.27	C <sub>7</sub> H <sub>15</sub> NaO <sub>3</sub> S · H <sub>2</sub> O			anionic
A3729	1-Hexanesulfonic acid sodium salt anhydrous	188.22	C <sub>6</sub> H <sub>13</sub> NaO <sub>3</sub> S			anionic
A1014	1-Hexanesulfonic acid sodium salt monohydrate	206.24	C <sub>6</sub> H <sub>13</sub> NaO <sub>3</sub> S · H <sub>2</sub> O			anionic
A1163	N-Lauroylsarcosine sodium salt	293.39	C <sub>15</sub> H <sub>28</sub> NNaO <sub>3</sub>	13.7 mM		anionic
A1385	Lithium dodecylsulfate (LiDS)	272.33	C <sub>12</sub> H <sub>25</sub> LiO <sub>4</sub> S	8.7 mM		anionic
A1386	MEGA-8	321.42	C <sub>15</sub> H <sub>31</sub> NO <sub>6</sub>	58 mM		nonionic
A3893	MEGA-9	335.44	C <sub>16</sub> H <sub>33</sub> NO <sub>6</sub>	25 mM		nonionic
A1017	1-Nonanesulfonic acid sodium salt	230.30	C <sub>9</sub> H <sub>19</sub> NaO <sub>3</sub> S			anionic
A1694	Nonidet® P40 (Substitute) Mixture of 15 homologues			0.34 mM	13.5	nonionic
A6742	n-Nonyl-β-D-glucopyranoside BioChemica	306.40	C <sub>15</sub> H <sub>30</sub> O <sub>6</sub>	18 - 20 mM		nonionic
A6814/A6768	n-Nonyl-β-D-maltoside	468.41	C <sub>21</sub> H <sub>40</sub> O <sub>11</sub>	6 mM		nonionic
A1016	1-Octanesulfonic acid sodium salt	216.28	C <sub>8</sub> H <sub>17</sub> NaO <sub>3</sub> S			anionic
A1010/A6813	n-Octyl-β-D-glucopyranoside	292.38	C <sub>14</sub> H <sub>28</sub> O <sub>6</sub>	25-30 mM		nonionic
A1145	n-Octyl-β-D-thioglucopyranoside	308.44	C <sub>14</sub> H <sub>28</sub> O <sub>5</sub> S	9 mM		nonionic
A6824	Octyl-D-glucopyranoside	292.38	C <sub>14</sub> H <sub>28</sub> O <sub>6</sub>			nonionic
A1709	1-Pentanesulfonic acid sodium salt anhydrous	174.20	C <sub>5</sub> H <sub>11</sub> NaO <sub>3</sub> S			anionic
A1013	1-Pentanesulfonic acid sodium salt monohydrate	192.12	C <sub>5</sub> H <sub>11</sub> NaO <sub>3</sub> S · H <sub>2</sub> O			anionic
A1288	Pluronic® F-68	~ 8350				nonionic
A4518	Saponin from Quillaja Bark					nonionic
A1112	SDS (Sodium dodecylsulfate)	288.38	C <sub>12</sub> H <sub>25</sub> NaO <sub>4</sub> S	8.2 mM	40.0	anionic

Prod.-No.	Description	M	Formula	CMC (25°C)	HLB	Class
A0979	Sodium cholate	430.57	C <sub>24</sub> H <sub>39</sub> NaO <sub>5</sub>	10 mM		anionic
A1531	Sodium deoxycholate	414.57	C <sub>24</sub> H <sub>39</sub> NaO <sub>4</sub>	2.7 mM (20°C)		anionic
A2225	Sucrose monolaurate	524.60	C <sub>24</sub> H <sub>44</sub> O <sub>12</sub>	0.4 mM		nonionic
A1460	Sulfobetaine SB 12	335.55	C <sub>17</sub> H <sub>37</sub> NO <sub>3</sub> S	3.3 mM		zwitterionic
A1162	Sulfobetaine SB 14	363.60	C <sub>19</sub> H <sub>41</sub> NO <sub>3</sub> S	0.3 mM		zwitterionic
A4810	n-Tetradecyl-β-D-maltoside	538.63	C <sub>26</sub> H <sub>50</sub> O <sub>11</sub>			nonionic
A6771	n-Tridecyl-β-D-maltoside	524.64	C <sub>25</sub> H <sub>48</sub> O <sub>11</sub>	0.033 mM		nonionic
A1388	Triton® X-100	646.85	C <sub>34</sub> H <sub>62</sub> O <sub>11</sub>	0.3 mM	13.5	nonionic
A3848	Triton® X-114	558.75	C <sub>30</sub> H <sub>54</sub> O <sub>9</sub>	0.35 mM	12.4	nonionic
A1389	Tween® 20	1227.72	C <sub>58</sub> H <sub>114</sub> O <sub>26</sub>	0.059 mM	16.7	nonionic
A1390	Tween® 80	1310		0.01 mM	15.0	nonionic
A6808/A6770	n-Undecyl-β-D-maltoside	496.59	C <sub>23</sub> H <sub>44</sub> O <sub>11</sub>	0.6 mM		nonionic

#### Abbreviations:

CHAPS (3-(3-Cholamidopropyl)-dimethylammonio-1-propanesulfonate)  
 CHAPSO (3-(3-Cholamidopropyl)-dimethyl-ammonio-2-hydroxy-1-propanesulfonate)  
 Deoxy-BIGCHAP (N,N-Bis-[3-(D-gluconamido)-propyl]-deoxycholamide)  
 HECAMEG (6-O-(N-Heptylcarbamoyl)-methyl-α-D-glucopyranoside)  
 MEGA-8 (Octanoyl-N-methylglucamide)  
 MEGA-9 (N-Nonanoyl-N-methylglucamide)  
 SDS (Sodium dodecylsulfate)  
 Sulfobetaine SB 12 (N-Dodecyl-dimethyl-3-ammonio-1-propanesulfonate)  
 Sulfobetaine SB 14 (N-Tetradecyl-dimethyl-3-ammonio-1-propanesulfonate)

#### Trademarks:

Brij (Atlas Chemicals Co.)  
 Nonidet (Shell)  
 Triton (Union Carbide Co.)  
 Pluronic, Tween (ICI America Inc.)

t e n s i d e s

# analysis

## Detergent Analysis

All of the carbohydrate-based detergents are crystallised to be greater than 99 % pure as measured by TLC, HPLC, double melting point and by the following analytical procedures.

### Measurement of purity (HPLC, TLC)

Each lot is first analysed by TLC and HPLC. All detergents are greater than 99 % pure. TLC was performed on silica gel (Merck 60 F(254), dichloromethane/methanol 7:1) and detection was effected by spraying the TLC plate with a solution of ethanol-sulfuric acid (9:1) followed by heating. HPLC was performed on a standard C18 column with an eluant of methanol/water. The ratio of methanol to water may vary from 65/35 to 85/15 depending on the hydrophobicity of the detergent. A 50 % solution of detergent in MeOH is injected into HPLC system. [P. Fromme, H. T. Witt, Improved isolation and crystallisation of Photosystem I for structural analysis; *BBA*, **1365** (1998) 175 - 184].

### Alcohol and $\beta$ -isomer contamination

$\beta$ -n-Alkylglycoside detergents are prepared by glycosylation of the hydrophobic alcohol with activated glucose or maltose acetates. Nearly all products are purified chromatographically and by crystallisation. Both alcohol and  $\beta$ -isomer are completely removed during the processing. Trace amounts of this alcohol in the detergent lot can cause cloudiness in the detergent solution. The  $\beta$ -anomeric purity of the detergent is advantageous especially for the crystallisation of membrane proteins.

### Measurement of purity (Double melting point)

The double melting properties of  $\beta$ -n-alkylglycosides have long been known (E. Fischer, B. Helferich; *Liebigs Ann. Chem.* **383**, 68 (1911). Alkylglycosides have liquid crystal properties and they can form mesophases in the melt (a state of order between crystals and liquids). In the smectic A mesophase the amphiphilic rod-like molecules form layered structures – a microscopic separation by a sequence of hydrophilic and lipophilic layers. In the isotropic phase the molecules are packed as a liquid in a random way. An optical polarised microscope equipped with a hotstage and a central processor is used to identify transition temperatures from the smectic A to the isotropic phase. Measurement of transition temperatures (double melting temperatures) gives a very sensitive test for the contamination of the crystalline detergent with hydrophobic alcohol,  $\beta$ -glycoside, solvents and others organic and anorganic impurities.

### Absorbance

Alkyl-glucosides and -maltosides have low absorbance throughout the UV region. MEGA detergents containing amido group have high absorbance at 230 nm. The absorbance of the 1 % w/v detergent solution in water is measured in the UV region.

### Fluorescence

A very low level of aromatic impurity can still result in a large fluorescence background. The fluorescence of the detergent solution is compared to a standard 0.1 % BSA solution. The excitation wavelength is 280 nm and the emission is measured at 345 nm.

### Conductance

Measurement of conductance gives a very sensitive test for the presence of ionic impurities. A solution of nonionic detergent should have conductance nearly the same as deionised water.

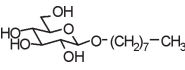
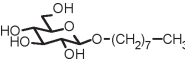
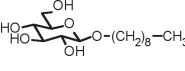
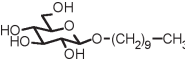
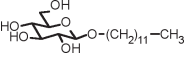
### Measurement of pH

The pH of the detergent solution at appropriate concentration is measured. The pH value of the solution should be neutral.

### Solubility in water

Some of the impurities (e.g. hydrophobic alcohol) in detergent preparations are not soluble in water. Therefore cloudiness of a detergent solution at a concentration where it is known to be soluble indicates the presence of an insoluble impurity.

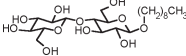
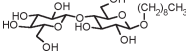
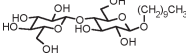
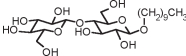
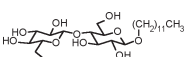
## Nonionic detergents

Alkyl β-D-glucopyranosides		Prod.-No.	Size
n-Hexyl β-D-glucopyranoside		A6815	on request
n-Heptyl β-D-glucopyranoside		A6807	on request
<b>n-Octyl β-D-glucopyranoside (OG) &gt; 99 % highly purified</b>		<b>A1010</b>	
	C <sub>14</sub> H <sub>28</sub> O <sub>6</sub> MW 292.38 CAS 29836-26-8, CMC (H <sub>2</sub> O) 18-20 mM		1 g
	White solid crystal, [α] <sub>D</sub> -34° (c 5; H <sub>2</sub> O)		5 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.03		10 g
	Solubility: >1 g in 10 ml H <sub>2</sub> O or EtOH clear, colourless solution		25 g
	Contaminants: n-Octanol <0.001 %, α-Isomer <0.01 % (HPLC)		Bulk > 1 kg
<b>n-Octyl β-D-glucopyranoside (OG) &gt; 99.5 % Crystallography grade</b>		<b>A6813</b>	
	C <sub>14</sub> H <sub>28</sub> O <sub>6</sub> MW 292.38 CAS 29836-26-8, CMC(H <sub>2</sub> O) 18-20 mM		1 g
	White solid crystal, [α] <sub>D</sub> -34° (c 5; H <sub>2</sub> O)		5 g
	thermal transition: 70.1°C (smectic A), 106.8°C (isotropic)		10 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.03		25 g
	Solubility: >1 g in 10 ml H <sub>2</sub> O or EtOH clear, colourless solution		Bulk > 1 kg
<b>n-Nonyl β-D-glucopyranoside &gt; 99 % highly purified</b>		<b>A6742</b>	
	C <sub>15</sub> H <sub>30</sub> O <sub>6</sub> MW 306.40 CAS 69984-73-2, CMC (H <sub>2</sub> O) 18-20 mM		1 g
	White solid crystal, [α] <sub>D</sub> -33° (c 5; H <sub>2</sub> O)		5 g
	thermal transition: 72.3°C (smectic A), 118.1°C (isotropic)		10 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.03		25 g
	Solubility: >1 g in 10 ml H <sub>2</sub> O or EtOH clear, colourless solution		Bulk > 250 g
<b>n-Decyl β-D-glucopyranoside (DG) &gt; 99 % highly purified</b>		<b>A3208</b>	
	C <sub>16</sub> H <sub>32</sub> O <sub>6</sub> MW 320.43 CAS 58846-77-8, CMC (H <sub>2</sub> O) 2.2 mM		1 g
	White solid crystal, [α] <sub>D</sub> -27° (c 1; EtOH)		5 g
	thermal transition: 75.2°C (smectic A), 130.0°C (isotropic)		10 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.03		25 g
	Solubility: >1 g in 10 ml H <sub>2</sub> O or EtOH clear, colourless solution		Bulk > 250 g
<b>n-Undecyl β-D-glucopyranoside &gt; 99.6 % highly purified</b>		<b>A6822</b>	on request
<b>n-Dodecyl β-D-glucopyranoside &gt; 99 % highly purified</b>		<b>A5890</b>	
	C <sub>18</sub> H <sub>36</sub> O <sub>6</sub> MW 348.48 CAS 59122-55-3		1 g
	White solid crystal, CMC (H <sub>2</sub> O) 0.19 mM [α] <sub>D</sub> -24.7° (c 1; EtOH)		5 g
	thermal transition: 77.2°C (smectic A), 137.4°C (isotropic)		10 g
	Absorbance (0.1 %, H <sub>2</sub> O, 260 nm): <0.03		25 g
	Solubility: >0.1 g in 10 ml H <sub>2</sub> O or EtOH clear, colourless solution		Bulk > 250 g
Contaminants: n-Dodecanol <0.001 %, α-Isomer <0.01 % (HPLC)			
Purity >99 % (HPLC)			

New procedures for the preparation of the anomeric pure alkyl-β-glycoside-detergents were developed. The α-Isomer is completely removed by chromatography. All of our detergents are crystallised to be greater than 99 % pure. The anomeric purity of the detergent is advantageous especially for the isolation and crystallisation of membrane proteins [P. Fromme, H. T. Witt, Improved isolation and crystallisation of Photosystem I for structural analysis; *Biochem. Biophys. Acta*, **1365** (1998) 175-184].

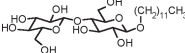
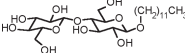
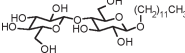
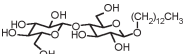
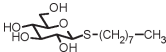
nonionic

## Nonionic detergents (continued)

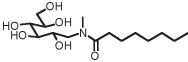
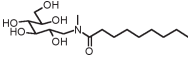
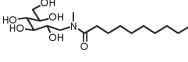
Alkyl $\beta$ -maltosides	Prod.-No.	Size
<b>n-Hexyl-<math>\beta</math>-maltoside</b>	<b>A6820</b>	<b>on request</b>
<b>n-Heptyl-<math>\beta</math>-maltoside</b>	<b>A6818</b>	<b>on request</b>
<b>n-Octyl-<math>\beta</math>-maltoside</b>	<b>A6809</b>	<b>on request</b>
<b>n-Nonyl <math>\beta</math>-maltoside (NM) &gt; 99 % highly purified</b>	<b>A6814</b>	
 <p> <math>C_{21}H_{40}O_{11}</math> MW 468.41 CMC (H<sub>2</sub>O) 6 mM                      White solid crystal, <math>[\alpha]_D^{+55^\circ}</math> (c 1; H<sub>2</sub>O)                      Absorbance (1 %, H<sub>2</sub>O, 280 nm): &lt;0.04, Aggregation No.: 55                      Solubility: 1 g/10 ml H<sub>2</sub>O, 21°C clear, colourless solution                      Contaminants: n-Nonanol &lt;0.001 % (HPLC), <math>\alpha</math>-Isomer &lt;0.01 %                      Purity &gt;99 % (HPLC)                 </p>		1 g 5 g 10 g 25 g Bulk > 250 g
<b>n-Nonyl <math>\beta</math>-maltoside (NM) &gt; 99.5 % Crystallography grade</b>	<b>A6768</b>	
 <p> <math>C_{21}H_{40}O_{11}</math> MW 468.41 CMC (H<sub>2</sub>O) 6 mM                      White solid crystal, <math>[\alpha]_D^{+55^\circ}</math> (c 1; H<sub>2</sub>O)                      thermal transition: 81°C (smectic A), 197°C (isotropic)                      Absorbance (1 %, H<sub>2</sub>O, 280 nm): &lt;0.04, Aggregation No.: 55                      Solubility: 1 g/10 ml H<sub>2</sub>O, 21°C clear, colourless solution                      Contaminants: n-Nonanol &lt;0.001 % (HPLC), <math>\alpha</math>-Isomer &lt;0.01 %                      Purity &gt;99.5 % (HPLC)                 </p>		1 g 5 g 10 g 25 g Bulk > 250 g
<b>n-Decyl-<math>\beta</math>-maltoside (DM) &gt; 99 % highly purified</b>	<b>A6816</b>	
 <p> <math>C_{22}H_{42}O_{11}</math> MW 482.57 CAS 82494-09-5, CMC (H<sub>2</sub>O) 1.8 mM                      White solid crystal, <math>[\alpha]_D^{+53.0^\circ}</math> (c 4; H<sub>2</sub>O)                      Absorbance (1 %, H<sub>2</sub>O, 260 nm): &lt;0.04                      Solubility: 1 g/10 ml H<sub>2</sub>O, 21°C clear, colourless solution                      Contaminants: n-Decanol &lt;0.001 % (HPLC), <math>\alpha</math>-Isomer &lt;0.01 %                      Purity &gt;99 % (HPLC)                 </p>		1 g 5 g 10 g 25 g Bulk > 250 g
<b>n-Decyl-<math>\beta</math>-maltoside (DM) &gt; 99.5 % Crystallography grade</b>	<b>A6769</b>	
 <p> <math>C_{22}H_{42}O_{11}</math> MW 482.57 CAS 82494-09-5, CMC (H<sub>2</sub>O) 1.8 mM                      White solid crystal, <math>[\alpha]_D^{+53.0^\circ}</math> (c 4; H<sub>2</sub>O)                      thermal transition: 89.2°C (smectic A), 208°C (isotropic)                      Absorbance (1 %, H<sub>2</sub>O, 260 nm): &lt;0.04;                      Solubility: 1 g/10 ml H<sub>2</sub>O, 21°C clear, colourless solution;                      Contaminants: n-Decanol &lt;0.001 % (HPLC), <math>\alpha</math>-Isomer &lt;0.01 %                      Purity &gt;99.5 % (HPLC)                 </p>		1 g 5 g 10 g 25 g Bulk > 250 g
<b>n-Undecyl-<math>\beta</math>-maltoside &gt; 99 % highly purified</b>	<b>A6808</b>	
 <p> <math>C_{23}H_{44}O_{11}</math> MW 496.59 CAS 170552-39-3, CMC (H<sub>2</sub>O) 0.6 mM;                      White solid crystal, <math>[\alpha]_D^{+50^\circ}</math> (c 1; H<sub>2</sub>O)                      Absorbance (1 %, H<sub>2</sub>O, 260 nm): &lt;0.04;                      Solubility: 1 g/10 ml H<sub>2</sub>O, 21°C clear, colourless solution;                      Contaminants: n-Undecanol &lt;0.001 % (HPLC), <math>\alpha</math>-Isomer &lt;0.01 %                      Purity &gt;99 % (HPLC)                 </p>		1 g 5 g 10 g 25 g Bulk > 250 g



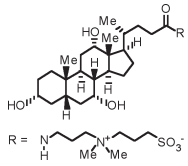
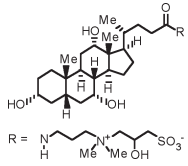
## Nonionic detergents (continued)

Alkyl $\beta$ -maltosides continued		Prod.-No.	Size
<b>n-Undecyl-<math>\beta</math>-maltoside &gt; 99.5 % Crystallography grade</b>		<b>A6770</b>	
	$C_{23}H_{44}O_{11}$ MW 496.59 CAS 170552-39-3, CMC (H <sub>2</sub> O) 0.59 mM;		1 g
	White solid crystal, $[\alpha]_D^{+50}$ (c 1; H <sub>2</sub> O)		5 g
	thermal transition: 96°C (smectic A), 236°C (isotropic)		10 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.04;		25 g
	Solubility: 1 g/10 ml H <sub>2</sub> O, 21°C clear, colourless solution;		Bulk > 250 g
	Contaminants: n-Undecanol <0.001 % (HPLC), $\alpha$ -Isomer <0.01 %		
	Purity >99.5 % (HPLC)		
<b>n-Dodecyl-<math>\beta</math>-maltoside (DDM) &gt; 99 % highly purified</b>		<b>A0819</b>	
	$C_{24}H_{46}O_{11}$ MW 510.63 CAS 69227-93-6, CMC (H <sub>2</sub> O) 0.17 mM		1 g
	White solid crystal, $[\alpha]_D^{+48}$ (c 1; H <sub>2</sub> O)		5 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.04, Aggregation No.: 98		10 g
	Solubility: 1 g/10 ml H <sub>2</sub> O, 21°C clear, colourless solution		25 g
	Contaminants: n-Dodecanol <0.001 % (HPLC), $\alpha$ -Isomer <0.01 %		Bulk > 250 g
	Purity >99 % (HPLC)		
<b>n-Dodecyl-<math>\beta</math>-maltoside (DDM) &gt; 99.5 % Crystallography grade</b>		<b>A6817</b>	
	$C_{24}H_{46}O_{11}$ MW 510.63 CAS 69227-93-6, CMC (H <sub>2</sub> O) 0.17 mM		1 g
	White solid crystal, $[\alpha]_D^{+48}$ (c 1; H <sub>2</sub> O)		5 g
	thermal transition: 103°C (smectic A), 246°C (isotropic)		10 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.04, Aggregation No.: 98		25 g
	Solubility: 1 g/10 ml H <sub>2</sub> O, 21°C clear, colourless solution		Bulk > 250 g
	Contaminants: n-Dodecanol <0.001 % (HPLC), $\alpha$ -Isomer <0.01 %		
	Purity >99.5 % (HPLC)		
<b>n-Tridecyl-<math>\beta</math>-maltoside (TDM) &gt; 99.5 % Crystallography grade</b>		<b>A6771</b>	
	$C_{25}H_{48}O_{11}$ MW 524.64 CMC (H <sub>2</sub> O) 0.033 mM		1 g
	White solid crystal, $[\alpha]_D^{+45.5}$ (c 1; H <sub>2</sub> O)		5 g
	thermal transition: 110°C (smectic A), 255°C (isotropic)		10 g
	Absorbance (1 %, H <sub>2</sub> O, 260 nm): <0.04, Aggregation No.: 105		25 g
	Solubility: 1 g/10 ml H <sub>2</sub> O, 21°C clear, colourless solution		Bulk > 100 g
	Contaminants: n-Tridecanol <0.001 % (HPLC), $\alpha$ -Isomer <0.01 %		
	Purity >99.5 % (HPLC)		
<b>Alkyl 1-thio-<math>\beta</math>-glycosides</b>			
<b>n-Octyl 1-thio-<math>\beta</math>-D-glucopyranoside</b>		<b>A1145</b>	
	$C_{14}H_{28}O_5S$ MW 308.44 CAS 85618-21-9 CMC (H <sub>2</sub> O) 9 mM		1 g
	White solid crystal, m.p. 128°C, $[\alpha]_D^{-53}$ (c 1; MeOH)		5 g
	Absorbance (0.5 %, H <sub>2</sub> O, 260 nm): <0.25		10 g
	Solubility: >8 g in 100 ml H <sub>2</sub> O clear, colourless solution		25 g
	Contaminants: n-Octanthiol <0.001 %, $\alpha$ -Isomer <0.01 % (HPLC)		Bulk > 1 kg
	Purity >99 % (HPLC)		
<b>n-Decyl 1-thio-<math>\beta</math>-D-glucopyranoside</b>		<b>A6819</b>	<b>on request</b>
<b>n-Dodecyl 1-thio-<math>\beta</math>-maltoside</b>		<b>A6821</b>	<b>on request</b>

## Nonionic detergents (continued)

<i>N</i> -( <i>n</i> -Alkanoyl)- <i>N</i> -methylglucamines - MEGA	Prod.-No.	Size
<b>MEGA-8 (<i>N</i>-Octanoyl-<i>N</i>-methylglucamin)</b>	<b>A1386</b>	
 <p> <math>C_{15}H_{31}NO_6</math> MW 321.42 CAS 85316-98-9            White solid CMC (<math>H_2O</math>) 79 mM            m.p. 88°C, <math>[\alpha]_D -17.7^\circ</math> (c 2; <math>H_2O</math>)            Absorbance (1 %, <math>H_2O</math>, 260 nm): &lt;0.01            Solubility: &gt;2 g/10 ml <math>H_2O</math>, 21°C clear, colourless solution            Purity (HPLC): &gt;99 %         </p>		1 g 5 g 10 g 25 g Bulk > 1 kg
<b>MEGA-9 (<i>N</i>-Nonanoyl-<i>N</i>-methylglucamin)</b>	<b>A3893</b>	
 <p> <math>C_{16}H_{33}NO_6</math> MW 335.44 CAS 85261-19-4            White solid crystal CMC (<math>H_2O</math>) 25 mM            m.p. 89°C, <math>[\alpha]_D -17.0^\circ</math> (c 2; <math>H_2O</math>)            Absorbance (1 %, <math>H_2O</math>, 260 nm): &lt;0.01            Solubility: &gt;2 g/10 ml <math>H_2O</math>, 21°C clear, colourless solution            Purity (HPLC): &gt;99 %         </p>		1 g 5 g 10 g 25 g Bulk > 1 kg
<b>MEGA-10 (<i>N</i>-Decanoyl-<i>N</i>-methylglucamin)</b>	<b>A4761</b>	
 <p> <math>C_{17}H_{35}NO_6</math> MW 349.47 CAS 85261-20-7            White solid crystal CMC (<math>H_2O</math>) 6 - 7 mM            m.p. 91° - 93°C, <math>[\alpha]_D -16.0^\circ</math> (c 1; <math>H_2O</math>)            Absorbance (1 %, <math>H_2O</math>, 260 nm): &lt;0.01            Solubility: &gt;1 g/10 ml <math>H_2O</math>, 21°C clear, colourless solution            Purity (HPLC): &gt;99 %         </p>		1 g 5 g 10 g 25 g Bulk > 1 kg

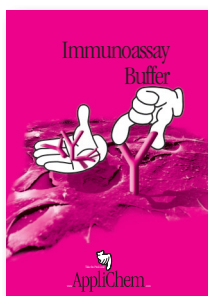
## Ionic detergents

CHAPS	Prod.-No.	Size
<b>3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane sulfonate</b>	<b>A1099</b>	
 <p> <math>C_{32}H_{58}N_2O_8S</math> MW 614.89 CAS 75621-03-3            White solid crystal CMC 4.2 - 6.3 mM Aggregation No.: 10            Absorbance (1 cm/5 %, <math>H_2O</math>) 290 nm &lt;0.5; 400 nm 0.02            Pb &lt;0.005 %; Na &lt; 0.005 %; N 4.5%; <math>H_2O</math> 2 %            Purity (HPLC): &gt;99 %            CHAPS is a zwitter-ionic detergent with non denaturing properties especially useful for solubilisation of membrane proteins used in a concentration of 1 - 13 mM.         </p>		5 g 10 g 25 g 50 g 100 g
<b>CHAPSO</b>		
<b>3-[(3-Cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propane sulfonate</b>	<b>A1100</b>	
 <p> <math>C_{32}H_{58}N_2O_8S</math> MW 630.87 CAS 82473-24-3            White solid crystal CMC 8 mM Aggregation No.: 11            Absorbance (1 cm/5 %, <math>H_2O</math>) 290 nm &lt;0.5; 400 nm 0.02            Purity (HPLC): &gt;99 %            CHAPSO is a zwitter-ionic detergent with non denaturing properties as CAPS or OG especially useful for the solubilisation of membrane proteins at a concentration of 1 %.         </p>		1 g 5 g 25 g



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## Immunoassay buffer

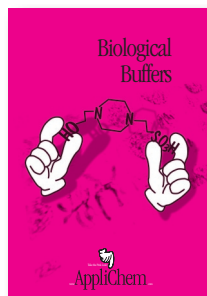
Anybody, who ever conducted an ELISA, RIA, Blot or other immunoassay knows: Many assays cannot be evaluated, because the background is too strong. This brochure informs about this problem and the products to resolve these problems.



## Safety First:

### Mycoplasma in cell cultures?

Many cell cultures are contaminated with mycoplasma. Apart from a control kit to prove the existence of mycoplasma contamination, we also offer the antibiotics for the treatment of cell cultures and reagents for the preventive cleaning of the CO<sub>2</sub> incubators and water baths.



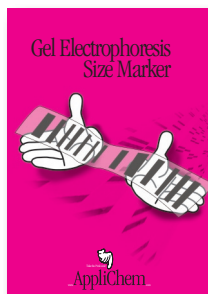
## Biological buffers

Basically no experiment exists, that does not employ any buffer substance. The "Biological Buffers" brochure offers an overview of the characteristics, the selection criteria and useful hints regarding the use of biological buffers.



## Transfer membranes

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