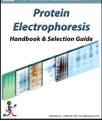


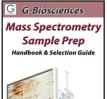
G G-Biosciences Protease & Phosphatase Inhibitors, Enzymes & Assays Handbook & **Selection Guide**



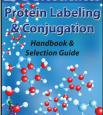
- Apoptosis Assays
- **Cytotoxicity Assays**
- SAM Methyltransferase Assays •
- **Protease Assays**
- **Phosphatase Assays Peroxide Assay** •
- **Protease Inhibitor Cocktails**
- **Individual Protease Inhibitors** •
- **Protease Assays** •
- **Proteases for Mass Spec.**

G-Biosciences

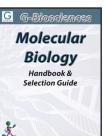












- Sequencing Grade Proteases •

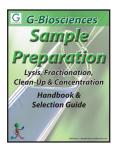
Gel Preparation Chemicals •

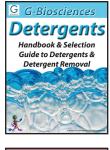
- Protein Marker Ladders •
- **Electrophoresis Buffers** •
- **Reducing & Alkylating Reagents** •
- **Protein Gel Stains** •
- **Protein Sample Preparation** •
- **Protein Clean-Up Systems**
- **Electrophoresis Reagents** •
 - Mass Spec Grade Protease
- **InGel Digestion Kits** •

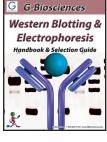
Biotin Labeling

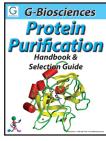
Peptide Generation Reagents •

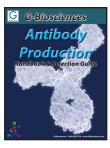
- Lysis Buffers & Systems
- **Protein Fractionation Kits**
- Dialysis (Micro) System
- Electrophoresis Clean-Up
- **Concentration Systems**
- **Contamination Removal**
- **Proteomic Grade Detergents**
- **Research Grade Detergents**
- Non-Ionic, Ionic & Zwitterionic •
- **Detergent Estimations**
- **Detergent Removal Systems**
- **1-Hour Western System**
- **Transfer Buffers & Membranes**
- Membrane Stains
- **Blocking Buffers**
- **Secondary Antibodies** ٠
- **Detection Reagents** •
- **Reprobing Reagents**
- Affinity Resins
- **6X His Protein Purification Kits**
- **GST Protein Purification Kits**
- **Antibody Purification** ٠
- **Activated Resins**
- **Buffers & Reagents**
- **Carrier Proteins**
- Peptide Coupling Systems
- **Antibody Purification Resins**
- **Antibody Fragmentation Kits**
- Homobifunctional
- Heterobifunctional
- **Optimizer Systems**
- **Cross-Linking Systems**
- **Apoptosis Assays**
- Cytotoxicity Assays
- SAM Methyltransferase Assays
- **Protease Assays**
- **Phosphatase Assays**
- **Peroxide Assay**
- **ELISA**

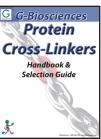


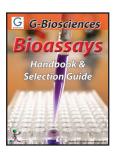














- Fluorescent Dye Labeling Kits
- **Enzyme Labeling Systems** •
- **Coated Plates** •
- **Blocking Buffers** •
- Wash Buffers •
- Secondary Antibodies
- **Detection Reagents** •
- Antibody Labeling Systems
- **DNA** Isolation
- **Transformation & Screening**
 - **Polymerase Chain Reaction** •
 - **Agarose Electrophoresis** •
 - **RNA** Isolation •
 - Yeast Transformation

•

•

•

•

Introduction

| Hydrophobicity | 2 |
|--|---|
| How do detergents work? | |
| How do detergents solubilize proteins? | 2 |
| Critical Micelle Concentration (CMC) | 3 |
| Classification & Characterization | 4 |
| Critical Micelle Concentration (CMC) | 4 |
| Kraft Point | 4 |
| Cloud Point | 4 |
| Aggregation Number | 4 |
| Hydrophile-Lipophile Balance (HLB) | 4 |
| | |

Proteomic Grade Detergents

Proteomic Grade Detergent Solutions (10%), Sterile4

Non Ionic Detergents

| Triton® X-100 | .5 |
|--|----|
| Triton® X-114 | .5 |
| Brij® 35 | .5 |
| Brij® 58 | |
| Tween® 20 | .6 |
| Tween® 80 | .6 |
| Nonidet® P-40 Substitute | .7 |
| Octyl β Glucoside | .7 |
| MEGA 8 | .7 |
| MEGA 10 | .8 |
| Big CHAP | .8 |
| Deoxy Big CHAP | .8 |
| Proteomic Grade Detergent Variety Pack | .8 |
| 2D-Detergent [™] | |
| | |

Ionic Detergents

| SDS | |
|--------------|--|
| СТАВ | |
| Deoxycholate | |

| Zwitterionic Detergents | 11 |
|---------------------------------|----|
| CHAPS | |
| CHAPSO | |
| Sulfobetaine 3-10 (SB 3-10) | |
| Sulfobetaine 3-12 (SB 3-12) | |
| Sulfobetaine 3-14 (SB 3-14) | |
| ASB-14 | |
| ASB-16 | |
| ASB-C8Ø | |
| Non-Detergent Sulfobetaine | |
| NDSB 201 | |
| Detergent Selection Guide | 14 |
| Detergent Removal | 16 |
| DetergentOUT [™] GBS10 | |

| OrgoSol DetergentOUT [™] | 17 |
|--------------------------------------|----|
| DetergentOUT [™] Tween® | |
| Detergent Optimization | 17 |
| Optimizer blueBALLS [™] | 17 |
| Detergent Assays | 18 |
| Detergent Assay | 18 |
| CMC-535 [™] Detergent Assay | |
| CIVIC-555 Detergent Assay | |
| SDS Detection & Estimation | |

Introduction

Detergents are amphipathic molecules that possess both a hydrophobic (water-fear) and a hydrophilic (water-friend) group that allow them to act as excellent solubilization agents.

Hydrophobicity

Hydrophobicity from the Greek words hydro (water) and phobos (fear) refers to the physical property of a molecule (known as a hydrophobe) that is repelled from a mass of water.

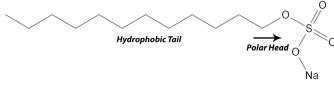
Water molecules form a highly ordered structure by the intermolecular action of its hydrogen bonds. Polar, or hydrophilic, molecules can readily dissolve in water as their charged groups can interact with the hydrogen bonds maintaining a ordered structure.

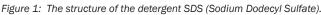
Non-polar, or hydrophobic, molecules are unable to form stable structures and are repelled by the water molecules and are therefore immiscible with the water. The addition of hydrophobic molecules disrupts the energy favoured structure of water, creating "holes" devoid of water molecules. The water molecules at the edge of the holes rearrange into an ordred manner and this results in an unfavorable decrease in entropy. To combat the loss of entropy, water molecules force the hydrophobic molecules to cluster to occupy the smallest space possible. This effect is known as the hydrophobic effect.

The hydrophobic effect plays an important role in protein structure and is involved in defining the tertiary structure of proteins. The amino acids of proteins can be polar or non-polar and therefore in an aqueous environment the proteins fold to protect the hydrophobic non-polar groups from the water molecules.

How do detergents work?

The structure of detergents is key to its ability to function as a solubilization agent. Detergent molecules contain a polar head group from which extends a long hydrophobic carbon tail.





The amphipathic properties of the detergent molecules allows them to exhibit unique properties in aqueous solutions. The polar (hydrophilic) head groups interact with the hydrogen bonds of the water molecules and the hydrophobic tails aggregate resulting in highly organized spherical structures called micelles. At low concentrations, the detergents exist as single molecules or small aggregates and as the concentration increases micelles begin to form. **The concentration at which micelles begin to form is known as the Critical Micelle Concentration (CMC).**

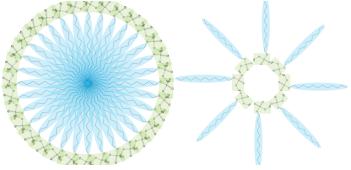


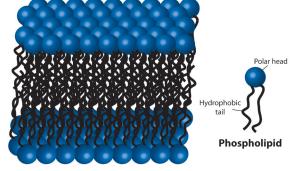
Figure 2: A detergent micelle formed with SDS molecules in an aqueous solution (left) or a non-aqueous solution (right).

Interestingly, detergents form reverse micelles in the presence of hydrocarbon solvents (non-aqueous solutions).

How do detergents solubilize proteins?

A wide range of detergents are routinely used to release, or solubilize, proteins from lipid membranes.

Biological membranes consist of phospholipids that are similar to detergents as they have the same amphipathic properties. The phospholipids have a charged polar head normally connected to two hydrophobic groups or tails. The phospholipids assemble as bilayers, with the hydrophobic tails between two faces of polar head groups.



Lipid Bilayer

Figure 3: The structure of a lipid bilayer and a phospholipid.

For biological membranes, proteins and lipids (i.e. cholesterol) are embedded in the bilayer forming the fluid mosaic model. The proteins are held in the lipid bilayer by hydrophobic interations between the lipid tails and hydrophobic protein domains. These integral membrane proteins are not soluble in aqueous solutions as they aggregate to protect their hydrophobic domains, but are soluble in detergent solutions.

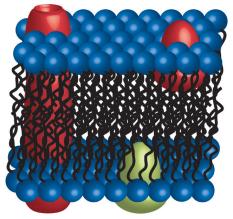


Figure 4: A Fluid -mosaic model of a biological membrane.

The proteins are released from lipid bilayers by detergents as the detergent micelles have similar properties as the lipid bilayer. The integral membrane proteins embed themselves in the detergent micelles protecting their hydrophobic domains from aggregation.

A schematic of how detergents solubilize membrane proteins is shown below. At low detergent concentrations, less than the detergent's CMC, the detergent molecules insert themselves in the lipid membrane and begin partioning the lipid bilayer. At concentrations equal to, or higher than the detergent's CMC, the lipid bilayer becomes saturated with detergent molecules and the lipid bilayer breaks apart. The resulting products are protein-detergent complexes, where the detergent hydrophobic regions bind to the protein hydrophobic domains protecting them from aggregations. In addition to these, detergent and detergent-lipid micelles are formed.

Introduction

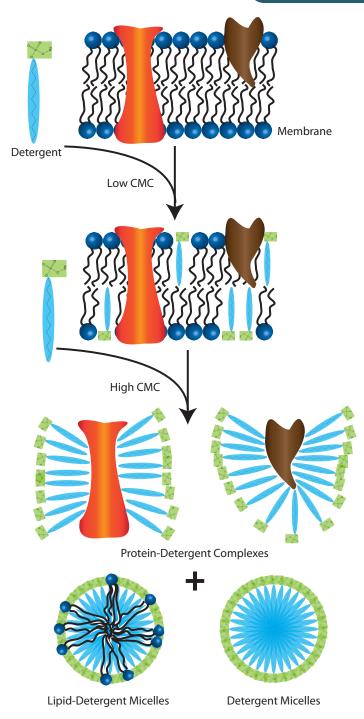


Figure 5: Schematic showing the stages of protein solubilization with detergent.

Critical Micelle Concentration (CMC)

The solubilization of proteins from lipid bilayers is dependent on the Critical Micelle Concentration (CMC) of the detergents.

The CMC is defined as the concentration of surfactants (detergents) above which micelles are spontaneously formed. The CMC is dependent on the alkyl chain length, presence of double bonds, branched points and additives in the solubilization buffers. As the alkyl chains increase, the CMC decreases; the introduction of double bonds and branch points increases the CMC; additives, such as urea, that are chaotropic increase the CMC.

The detergent CMC is important as it allows researcher's to use the precise amount of detergent, too little means inadequate solubilization of proteins, too much can affect downstream process and problematic detergent removal steps.

CMC can be determined by light scattering (increases with detergent concentration), surface tension (decrease) and dye solubilization (increase) (Vulliez-Le Normand and Jean-Luc Eisele (1993)). All three techniques are time consuming and are rarely performed for this reason. G-Biosciences has developed OptimizerblueBALLS[™], which is based on the dye solubilization method, but is significantly more convenient. More information on OptimizerblueBALLS[™] is provided later in this handbook.

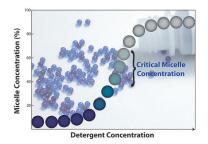


Figure 6: Graphical representation of critical micelle concentration determination. Blue colored Optimizer blueBALLS[™] imitate membrane proteins and solubilize when the critical micellar concentration is reached, releasing a non-reactive blue color into the extraction buffer.

Proteomic Grade Detergents

CLASSIFICATION & CHARACTERIZATION

There are a vast number of detergents available for protein solubilization. They can be classified based on the nature of their hydrophilic head group. The three classifications are:

- Non ionic
- Ionic
- Zwitterionic

In addition to the above classification, there are important properties or characteristics of detergents that can be used to aid researchers in their choice of detergent.

Critical Micelle Concentration (CMC)

The CMC is defined as the concentration of surfactants (detergents) above which micelles are spontaneously formed. See previous section.

Kraft Point

The Kraft Point is used to describe the temperature at which an equilibrium exist between an insoluble crystalline state, monomeric detergent and detergent micelles. At low temperatures, detergents form insoluble crystalline states that shift to detergent monomers and finally detergent micelles with increasing temperatures.

The temperature at which the CMC concentration is reached is known as the critical micellar temperature (CMT). In most cases, the CMT is equal to the Kraft Point.

Cloud Point

The Cloud Point is another temperature related property that is specific for non ionic detergents. As temperatures pass the CMT, the non ionic detergents become cloudy and separate into a detergentrich and an aqueous layer, a process known as phase separation. This temperature is known as the cloud point.

This property is used for the purification of integral membrane proteins with Triton[®] X-114. The cloud point of Triton[®] X-114 is 23 °C, therefore cellular membranes can be solubilized at 0 °C and then warmed to >23 °C. The integral membrane proteins partition into the detergent-rich phase away from the hydrophilic proteins that remain in the aqueous phase (Bordier, C (1981)).

Aggregation Number

This is quite simply the number of detergent molecules that are associated together to form a micelle and is calculated by:

Aggregation No. = <u>Micellar molecular weight</u> Monomeric molecular weight

The micellar molecular weight can be determined by gel filtration, sedimentation equilibrium, X-ray scattering or light scattering.

Hydrophile-Lipophile Balance (HLB)

A measure of the hydrophilic character of a detergent. Basically, detergents with HLB of 12-20 are preferred for non-denaturing solubilization; >20 for extrinsic protein solubilization. Detergents with a low HLB are more readily removed by hydrophobic chromatography as they are more hydrophobic.

Proteomic Grade Detergent Solutions (10%), Sterile

Ultra low carbonyl & peroxide contaminants

Many commercial grade detergents contain elevated levels of sulfhydryl oxidizing agents, peroxides, salts and carbonyl compounds. The proteins that are isolated with these detergents are highly susceptible to contaminating peroxides and carbonyls. The peroxides will oxidize proteins and the carbonyl groups will form Schiff's bases with the proteins that will interfere with a protein's structure.

Our Proteomic Grade Detergent Solutions contain reduced peroxides and carbonyl compounds. In addition, the detergents have less than 50μ S conductivity. These detergents are offered as sterile, 10% aqueous solutions, sealed under inert gas and are suitable for protein applications. These non ionic detergents are suitable for isolating membrane-protein complexes.

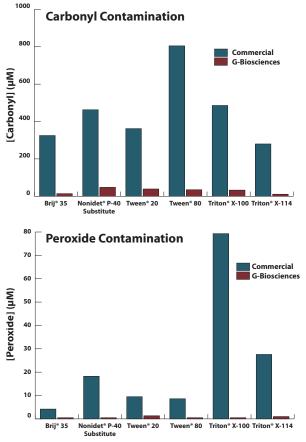


Figure 7: Comparison of aldehyde (top) and peroxide (bottom) concentration in G-Biosciences Proteomic Grade Detergent Solutions and non-proteomic grade commercially available detergents.

FEATURES

- Low peroxide contamination
- · Low carbonyl contamination
- · Low conductivity
- Sterile detergent solutions
- Reduced metal ions
- 10% aqueous solutions
- Sealed under inert gas to prevent oxidation

We offer a selection of widely used Proteomic Grade Detergent Solutions. The aldehyde and peroxide levels are <100 μ M and <50 μ M respectively with a conductivity of <50 μ S.

Non ionic detergents have a hydrophilic head group that is uncharged and are preferred for their ability to break lipid-lipid and lipid-protein interactions. They have limited ability to break proteinprotein interactions and are often referred to as non-denaturing detergents and are used to isolate biologically active membrane proteins.

The non ionic deteregents are supplied as a general Research Grade, Proteomic Grade (PG) Solutions and 2D-Detergents[™]. The Proteomic Grade (PG) Solutions have ultra low aldehvde (<100µM) and peroxide (<50µM) concentrations to reduce the effects of peroxidase and carbonyl compounds that negatively interact with membrane proteins. The 2D-Detergents[™] have low conductivity (<10µS) and ultra low aldehyde (<100µM) and peroxide (<50µM) concentrations.

Triton[®] X-100

Octylphenolpoly(ethyleneglycolether)

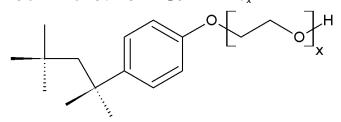


Figure 8: Structure of Triton® X-100.

Type: Non ionic detergent

Form: 10% aqueous solution (w/v) or 100% solution

Mol. Formula: $C_{34}H_{62}O_{11}$ for x =10

Mol. Weight: 647 (for x=10)

Absorbance (254nm): 0.16 (0.05% w/v)

Aldehyde content: <100µM

Peroxide content (as H₂O₂): <50µM

Critical micelle concentration (CMC): approx 0.2 x 10⁻³M (25°C) Aggregation number: 100-155

Cloud Point: 65°C

Average micellar weight: 80,000

Application: One of the most commonly used non ionic detergents for solubilizing membrane proteins during isolation of membraneprotein complexes. Ultra low aldehyde and peroxide concentrations reduce the effects of peroxidase and carbonyl compounds that negatively interact with membrane proteins

CITED REFERENCES

- Baindara, P. et al (2017) Sci Rep. DOI: 10.1038/srep46541
- Callif, B. I. et al (2017) Mol Cell Neurosci.http://dx.doi.org/10.1016/j.mcn.2017.01.003 2.
- Shane, M. W. et al (2016) AoB Plants. doi: 10.1093/aob/mcw040 З.
- 4. Nagy, S. et al (2016) Acta Vet Hung. 64:1 5.
- Baindara, P. et al (2015) Antimicrob. Agents. Chemother. DOI: 10.1128/AAC.01813-15 6. Simpson, M.T. et al (2015) 68:272
- 7.
- Kakasi, B. et al (2015) Acta Vet Hung. 63:118 8. Singh, P. K. et al (2014) FEBS J. 282:203
- 9. Portugal, G.S. et al (2014) J. Neurosci, 34:527
- 10 Harper, J. et al (2013) Method Mol. Biol. 1045:41
- Drury-Stewart, D. et al (2013) Stem Cell Res. Ther. 4:93 11
- Bonnet, S.A. et al (2013) J. Neurosci.33: 13398 Krejci, M.R. et al (2011) J. Struc. Biol. 176:192 12. 13.
- 14. Prado, I. et al (2009) Method Mol. Biol. 525:517
- 15. Cawley, N.X (2010) AM J Physiol Endocrinol Metab. 299:E189

| Cat. No. | Description | Size |
|----------------|--|-----------------|
| <u>786-513</u> | Triton [®] X-100, 100% solution | 500ml |
| <u>786-514</u> | Triton [®] X-100, 100% solution | 1L |
| DG007 | Triton [®] X-100, 10% solution | 5 x 10ml vials |
| DG008 | Triton [®] X-100, 10% solution | 10 x 10ml vials |
| <u>DG507</u> | Triton [®] X-100, 10% solution | 50ml bottle |
| <u>DG517</u> | Triton [®] X-100, 10% solution | 100ml bottle |
| | | |

Triton[®] X-114

Polyethylene glycol tert-octylphenyl ether

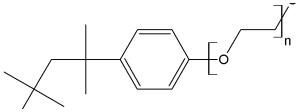


Figure 9: Structure of Triton® X-114.

Type: Non ionic detergent Form: 10% aqueous solution (w/v) **Mol. Formula:** $C_{14}H_{22}O \bullet [C_2H_4O]_{7.8}$ for n =8 **Mol. Weight:** ~537 (for n=7-8) Absorbance (254nm): 0.18 (0.05% w/v) Aldehyde content: < 100µM Peroxide content (as H₂O₂): < 50µM Critical micelle concentration (CMC): approx 0.35 x 10⁻³M (25°C) Cloud Point: 23°C

Application: A non ionic detergent with a low cloud point (23°C) making it suitable for protein solubilization with phase-partitioning of hydrophilic proteins from amphiphilic proteins

| Cat. No. | Description | Size |
|----------------|--|-----------------|
| <u>786-515</u> | Triton [®] X-114, 100% solution | 500ml |
| <u>786-516</u> | Triton [®] X-114, 100% solution | 1L |
| DG009 | Triton [®] X-114, 10% solution | 5 x 10ml vials |
| DG010 | Triton [®] X-114, 10% solution | 10 x 10ml vials |
| DG509 | Triton [®] X-114, 10% solution | 50ml bottle |
| <u>DG518</u> | Triton [®] X-114, 10% solution | 100ml bottle |

Brii® 35

Polyoxyethylene (23) lauryl ether

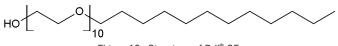


Figure 10: Structure of Brij® 35.

Type: Non ionic detergent

Form: 10% aqueous solution (w/v), white solid **Mol. Formula:** $C_{12}H_{26}O(OCH_2CH_2)_{10}$ Mol. Weight: 627 Absorbance (225nm): 0.07 (1% w/v) Aldehyde content: < 100µM Peroxide content (as H₂O₂): < 50µM Critical micelle concentration (CMC): 90µM Aggregation number: 24-40

Cloud Point: >100°C

Average micellar weight: 48,000

Appearance: Clear solution with a faint yellow color

Application: For protein extraction, permeabilization of cells, and preparation of yeast spheroplasts

CITED REFERENCES

Spoonamore, J. (2011) In: Probe Reports from the NIH Molecular Libraries Program. Available from: http://www.ncbi.nlm.nih.gov/books/NBK133418/

| Cat. No. | Description | Size |
|----------------|------------------------------------|-----------------|
| <u>786-351</u> | <u>Brij[®] 35</u> | 250g |
| <u>786-521</u> | <u>Brij[®] 35</u> | 500g |
| DG003 | Brij® 35, 10% solution | 5 x 10ml vials |
| DG004 | Brij [®] 35, 10% solution | 10 x 10ml vials |
| <u>DG503</u> | Brij [®] 35, 10% solution | 50ml bottle |
| DG515 | Brij [®] 35, 10% solution | 100ml bottle |

Brij[®] 58

Polyoxyethylene (20) cetyl ether

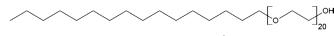


Figure 11: Structure of Brij® 58.

Type: Non ionic detergent Form: 10% aqueous solution (w/v), white solid Mol. Formula: $C_{16}H_{33}(OCH_2CH_2)_{20}$ -OH Mol. Weight: 1122 Absorbance (225nm): 0.0788 (1% w/v) Aldehyde content: < 100 μ M Peroxide content (as H_2O_2): < 50 μ M Critical micelle concentration (CMC): 7-77 μ M Aggregation number: 70 Cloud Point: >100°C Average micellar weight: 79,000 Appearance: Clear solution with a faint yellow color Application: For protein extraction, permeabilization of cell

Application: For protein extraction, permeabilization of cells, and preparation of yeast spheroplasts

| Cat. No. | Description | Size |
|----------------|------------------------------------|-----------------|
| <u>786-352</u> | <u>Brij[®] 58</u> | 250g |
| <u>786-522</u> | <u>Brij[®] 58</u> | 500g |
| DG005 | Brij [®] 58, 10% solution | 5 x 10ml vials |
| <u>DG006</u> | Brij [®] 58, 10% solution | 10 x 10ml vials |
| <u>DG505</u> | Brij [®] 58, 10% solution | 50ml bottle |
| DG516 | Brij [®] 58, 10% solution | 100ml bottle |

Tween[®] 20

Polyethylene glycol sorbitan monolaurate

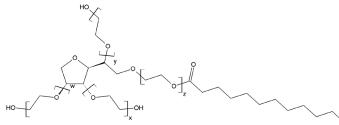


Figure 12: Structure of Tween® 20.

Type: Non ionic detergent Form: 10% aqueous solution (w/v), 100% solution Mol. Formula: $C_{18}H_{34}O_6 \cdot [C_2H_4O]_{w+x+y+z}$ for w+x+y+z =20 Mol. Weight: ~1227 (for w+x+y+z =20) Absorbance (215nm): 0.05 (0.05% w/v) Aldehyde content: < 100µM Peroxide content (as H₂O₂): < 50µM Critical micelle concentration (CMC): approx 0.06 x 10⁻³M Cloud Point: 76 °C

Application: A commonly used non ionic detergent for solubilizing membrane proteins during isolation of membrane-protein complexes

CITED REFERENCES

1. Luke, C. et al (2015) ACS Synth Biol. DOI: 10.1021/acssynbio.5b00094

| Cat. No. | Description | Size |
|----------------|--------------------------------------|-----------------|
| <u>786-517</u> | Tween [®] 20, 100% solution | 500ml |
| <u>786-518</u> | Tween® 20, 100% solution | 1L |
| <u>DG011</u> | Tween [®] 20, 10% solution | 5 x 10ml vials |
| DG012 | Tween [®] 20, 10% solution | 10 x 10ml vials |
| <u>DG511</u> | Tween [®] 20, 10% solution | 50ml bottle |
| <u>DG519</u> | Tween [®] 20, 10% solution | 100ml bottle |

Tween[®] 80

Polyethylene glycol sorbitan monooleate

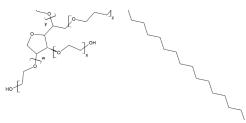


Figure 13: Structure of Tween® 80.

Type: Non ionic detergent

Form: 10% aqueous solution (w/v), 100% solution Mol. Formula: $C_{24}H_{46}O_6 \cdot [C_2H_4O]_{w+x+y+z}$ for w+x+y+z =20 Mol. Weight: ~1325 (for w+x+y+z =20) Absorbance (250nm): 0.14 (0.05% w/v) Aldehyde content: < 100µM Peroxide content (as H_2O_2): < 50µM Critical micelle concentration (CMC): ~0.012 x 10⁻³M (25°C) Aggregation number: 60 Cloud Point: 65°C

Average micellar weight: 79,000

Application: For solubilizing membrane proteins during isolation of membrane-protein complexes

CITED REFERENCES

6.

- O'Donnell, V. et al (2016) J Virol. doi: 10.1128/JVI.01760-16
- 2. Carlson, J. et al (2016) Viruses doi:10.3390/v8100291
- O'Donnell, V. (2016) Virus Res.doi:10.1016/j.virusres.2016.05.014
 Andrade-Ochoa, S. et al (2015) BMC Complement. Altern. Med. 15:332
- Andrade-Ochoa, S. et al (2015) BMC Complement. Altern. Med. 15:332
 Knudson, S.E. et al (2014) Tuberculosis. http://dx.doi.org/10.1016/j.tube.2014.03.007
 - Hewitt, D. et al (2008) J. Chromatogr A. 1215:156

| Cat. No. | Description | Size |
|----------------|--------------------------------------|-----------------|
| <u>786-519</u> | Tween® 80, 100% solution | 500ml |
| <u>786-520</u> | Tween [®] 80, 100% solution | 1L |
| DG013 | Tween [®] 80, 10% solution | 5 x 10ml vials |
| <u>DG014</u> | Tween [®] 80, 10% solution | 10 x 10ml vials |
| <u>DG513</u> | Tween [®] 80, 10% solution | 50ml bottle |
| <u>DG520</u> | Tween [®] 80, 10% solution | 100ml bottle |

Nonidet[®] P-40 Substitute

Nonylphenyl-polyethylene glycol

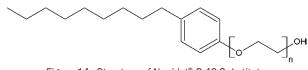


Figure 14: Structure of Nonidet® P-40 Substitute.

Type: Non ionic detergent

Form: 10% aqueous solution (w/v), 100% solution Mol. Formula: $C_{15}H_{24}O[C_2H_4O]_n$ Mol. Weight: 573 (for n=8)

Absorbance (254nm): 0.14 (0.05% w/v)Aldehyde content: < 100μ M

Peroxide content (as H₂O₂): < 50µM

Critical micelle concentration (CMC): approx 0.05-0.3mM (25°C) **Application:** A commonly used non ionic detergent for solubilizing membrane proteins during isolation of membrane-protein complexes

| Cat. No. | Description | Size |
|----------------|---|-----------------|
| <u>786-511</u> | Nonidet [®] P-40 Substitute, 100% solution | 500ml |
| <u>786-512</u> | Nonidet [®] P-40 Substitute, 100% solution | 1L |
| DG001 | Nonidet [®] P-40 Substitute, 10% solution | 5 x 10ml vials |
| DG002 | Nonidet [®] P-40 Substitute, 10% solution | 10 x 10ml vials |
| DG501 | Nonidet [®] P-40 Substitute, 10% solution | 50ml bottle |
| DG514 | Nonidet [®] P-40 Substitute, 10% solution | 100ml bottle |

Octyl β Glucoside

N-Octyl-beta-D-glucopyranoside

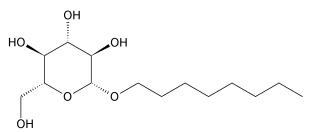


Figure 15: Structure of Octyl-β-Glucoside.

Type: Non ionic detergent Mol. Formula: C₁₄H₂₈O₆ Mol. Weight: 292.4 Form: White to off white powder Purity: >98% Solubility: Water soluble Critical micelle concentration (CMC): 20-25mM (25°C) Aggregation number: 84

Cloud Point: >100°C

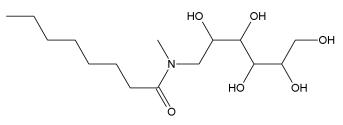
Average micellar weight: 25,000

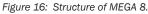
Application: Widely used for membrane proteins. For solubilization of membrane-bound proteins in their native state, and for preparation of lipid vesicles. Low molecular weight permits easy removal by dialysis. Useful for solubilizing enzymes, receptors and phosphatidylcholine bilayers

| Cat. No. | Description | Size |
|----------|-------------------|------|
| DG015 | Octyl β Glucoside | 1g |
| DG016 | Octyl β Glucoside | 5g |

MEGA 8

Octanoyl-N-methylglucamide





Type: Non ionic detergent Mol. Formula: C₁₅H₃₁NO₆ Mol. Weight: 321.4 Form: White powder Purity: >99% Solubility: Water soluble

Critical micelle concentration (CMC): 58mM (25°C) Application: Non ionic detergent that is water soluble and readily removed by dialysis. Used for the solubilization of membranes

CITED REFERENCES

Kudlinzki, D. et al (2015) Acta Cryst. 71:1088

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG017</u> | MEGA 8 | 1g |
| DG018 | MEGA 8 | 5g |

MEGA 9

Nonaoyl-N-methylglucamide

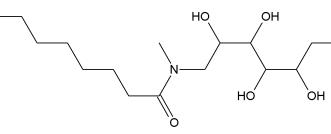


Figure 17: Structure of MEGA 9.

Type: Non ionic detergent Mol. Formula: C₁₆H₃₃NO₆ Mol. Weight: 335.4 Form: White powder Purity: >99% Solubility: Water soluble

Critical micelle concentration (CMC): 19-25mM (25°C) **Application:** Non ionic detergent that is water soluble and readily removed by dialysis. Used for the solubilization of membranes

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG019</u> | MEGA 9 | 1g |
| <u>DG020</u> | MEGA 9 | 5g |

MEGA 10

Decanoyl-N-methylglucamide

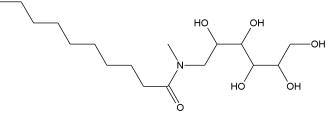


Figure 18: Structure of MEGA 10.

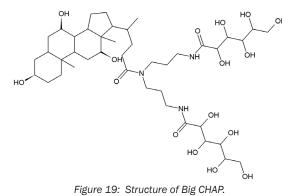
Type: Non ionic detergent **Mol. Formula:** C₁₇H₃₅NO₆ **Mol. Weight:** 349.5 **Form:** White powder **Purity:** >99% **Solubility:** Water soluble

Critical micelle concentration (CMC): 6-7mM (25°C) **Application:** Non ionic detergent that is water soluble and readily removed by dialysis. Used for the solubilization of membranes

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG021</u> | MEGA 10 | 1g |
| DG022 | MEGA 10 | 5g |

Big CHAP

N,N-Bis[3-(D-gluconamido)propyl]cholamide



Type: Non ionic detergent Mol. Formula: $C_{42}H_{75}N_3O_{16}$ Mol. Weight: 878.1 Form: Fine colorless crystals Purity: >99% Solubility: Water soluble Conductivity: <25µS in a 10% solution Critical micelle concentration (CMC): 3.4mM (25°C) Aggregation number: 10 Average micellar weight: 8800

Application: Non ionic detergent for membrane solubilization

| Cat. No. | Description | Size |
|----------|-----------------|------|
| DG023 | Big CHAP | 1g |
| DG024 | Big CHAP | 5g |

Deoxy Big CHAP

N,N-Bis[3-(D-gluconamido)propyl]deoxycholamide

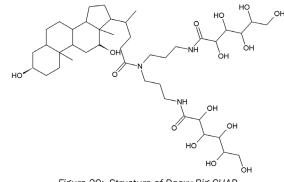


Figure 20: Structure of Deoxy Big CHAP.

Type: Non ionic detergent Mol. Formula: $C_{42}H_{75}N_3O_{15}$ Mol. Weight: 862.1 Form: White powder Purity: >95% Solubility: Water soluble Critical micelle concentration (CMC): 1.1-1.4mM (25°C) Aggregation number: 8-16

Average micellar weight: 10,500

Application: Non ionic detergent that is water soluble and has increased solubility compared to CHAPS. Used for the solubilization of membranes

| Cat. No. | Description | Size |
|--------------|----------------|------|
| DG025 | Deoxy Big CHAP | 1g |
| <u>DG026</u> | Deoxy Big CHAP | 5g |

Proteomic Grade Detergent Variety Pack

The variety pack contains a selection of our non ionic Proteomic Grade Detergent Solutions, Zwitterionic and non-detergent sulfobetaines for trial and optimization.

The following proteomic grade detergents are available as a trial pack. The pack contains one 10ml vial of 10% aqueous solutions of:

- Triton[®] X-100
- Triton[®] X-114
- Tween[®] 20
- Tween[®] 80
- Nonidet[®] P-40 Substitute
- Brij[®] 35
- Brij[®] 58

And 1gm of:

- CHAPS
- NDSB 201

 Cat. No.
 Description
 Size

 DG521
 Proteomic Grade Detergent Variety Pack
 9 vials

2D-Detergent[™]

Ultra low conductivity & low carbonyl & peroxide contaminants

Our 2D-Detergent[™] solutions contain reduced peroxides and carbonyl compounds. In addition, the detergents have less than 15µS conductivity. These detergents are offered as 10% aqueous solutions, sealed under inert gas and are suitable for all protein applications, including 2D-electrophoresis. These non ionic detergents are suitable for isolating membrane-protein complexes.

The aldehyde levels are <50 μ M, the peroxide levels are <10 μ M and have a conductivity of <15 μ S.

FEATURES

- Low conductivity; <15µS
- · Low peroxide contamination
- Low carbonyl contamination
- Reduced metal ions
- Ready to use 10% aqueous solutions
- · Sealed under inert gas to prevent oxidation

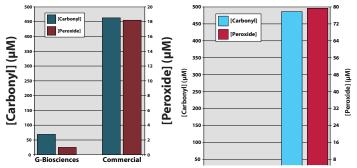


Figure 21: Comparison of carbonyl (as a measure of aldehyde) (blue) and peroxide (red) concentration in G-Biosciences 2D-Detergent[™] NP-40 Substitute (left) or 2D-Detergent[™] Triton[®] X-100 (right) and non-proteomic grade commercially available detergents.

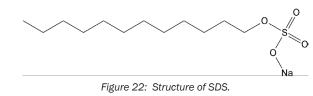
| Cat. No. | Description | Size |
|--------------|--|-----------------|
| <u>DG907</u> | <u>2D-Detergent[™] Triton[®] X-100</u> | 5 x 10ml vials |
| <u>DG908</u> | 2D-Detergent [™] Triton [®] X-100 | 10 x 10ml vials |
| <u>DG901</u> | 2D-Detergent [™] Nonidet [®] P-40 Substitute | 5 x 10ml vials |
| <u>DG902</u> | 2D-Detergent [™] Nonidet [®] P-40 Substitute | 10 x 10ml vials |

Ionic Detergents

lonic detergents are used for complete disruption of cellular structure and denaturing of proteins for separation during gel electrophoresis. Ionic detergents bind with protein molecules masking their native charge and rendering the protein molecules the overall charge of the ionic detergent.

SDS

Sodium dodecyl sulfate



Type: Anionic detergent Mol. Formula: C₁₂H₂₅NaO₄S

Mol. Weight: 288.38

Form: White to off white powder, 10% or 20% solution **Purity: >99%** Solubility: Water

Critical micelle concentration (CMC): 7-10mM (25°C)

Aggregation number: 62

Cloud point: >100°C

Average micellar weight: 18,000

Application: Capable of almost complete disruption of cellular structures and denaturation. Used for solubilization of a wide variety of proteins, including membrane proteins, for electrophoretic separation. Detergent molecules tightly bind with the protein molecules masking their native charge and rendering the protein molecules with an overall negative charge.

| Cat. No. | Description | Size |
|----------------|-------------------|-------|
| DG092 | <u>SDS</u> | 100g |
| <u>DG093</u> | <u>SDS</u> | 500g |
| <u>R014</u> | SDS, 10% Solution | 100ml |
| <u>786-016</u> | SDS, 20% Solution | 500ml |
| <u>786-017</u> | SDS, 20% Solution | 1L |

CTAB

Hexadecyltrimethylammonium bromide

Figure 23: Structure of CTAB.

Type: Cationic detergent **Mol. Formula:** $CH_3(CH_2)_{15}N(Br)(CH_3)_3$ Mol. Weight: 364.5 Form: White to off white powder **Purity: >99%** Solubility: Water soluble Critical micelle concentration (CMC): 1mM (25°C) Aggregation number: 61 in water; 169 in 13mM KBr Average micellar weight: 62,000 Application: A cationic detergent used for solubilization of a wide

CITED REFERENCES

- Canbek, Z.C. et al (2015) Cryst. Growth Des. DOI: 10.1021/acs.cgd.5b00121 2.
 - Youssry, M. et al (2012) Meas. Sci. Technol. 23:125306

variety of proteins and nucleic acids

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG094</u> | <u>CTAB</u> | 25g |
| <u>DG095</u> | <u>CTAB</u> | 100g |

Deoxycholate

Deoxycholic acid, sodium salt

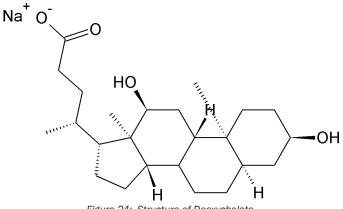


Figure 24: Structure of Deoxycholate.

Type: Anionic detergent Mol. Formula: C₂₄H₃₉NaO₄ Mol. Weight: 414.6 Form: White to off white powder

Purity: >99%

Solubility: Water soluble

Critical micelle concentration (CMC): 4-8mM (25°C) Aggregation number: 3-12

Average micellar weight: 1,200-5,000

Application: Anionic detergent useful for extraction of membrane proteins and nuclei isolation. Not recommended for use with Mn2+

| Cat. No. | Description | Size |
|--------------|---------------------------|------|
| <u>DG090</u> | Deoxycholate, sodium salt | 100g |
| <u>DG091</u> | Deoxycholate, sodium salt | 500g |

Zwitterionic Detergents

Zwitterionic detergents protect the native state of proteins without altering the native charge of the protein molecules. Zwitterionic detergents are used for isoelectric focusing and 2D electrophoresis. Synthetic zwitterionic detergents are known as sulfobetaines. Sulfobetaines retain their zwitterionic characteristics over a wide range of pH. The following zwitterionic detergents are the most efficient and widely used for 2D gel electrophoresis.

CHAPS

3-[(3-Cholamidopropyl)dimethylammonio]-1propanesulfonate

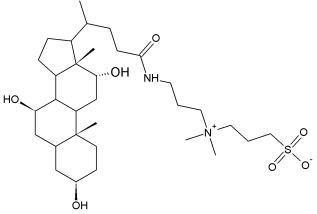


Figure 25: Structure of CHAPS.

Type: Zwitterionic detergent Mol. Formula: C₃₂H₅₈N₂O₇S Mol. Weight: 614.9 Form: White solid **Purity: >99%** Solubility: Water soluble Conductivity: <25µS in a 10% solution Critical micelle concentration (CMC): 6-10mM (25°C)

Aggregation number: 10

Cloud point: >100°C

Average micellar weight: 6150

Application: Zwitterionic detergent. Non-denaturing. Electrically neutral. CHAPS has all the advantages of sulfobetaine containing detergents: hydrophobic, bile salt, and anionic detergents in a single molecule. Better at solubilizing proteins and breaking protein-protein interactions. Less protein aggregation than non ionic detergents. Capable of solubilizing opiate receptors. CHAPS can be removed from protein solutions with a detergent removing gel or by dialysis.

CITED REFERENCES

- Kaliszewski, M. et al (2016) SOD1 Front. Cell. Neurosci. doi.10.3389/fncel.2016.00287 1.
- 2. Chandrasekhar, R. et al (2016) J Biol Chem. Doi: jbc.M115.705301.
- 3 Alzayady, K.J. et al (2013) JBC. 288:11122
- Alzayady, K.J. et al (2013) JBC. 288:29772 Onodera, H. et al (2013) Brain Res. 1534:87 4 5.
- 6. Ahmed, Z. et al (2013) Food Chem. 140:238
- 7.
- Song, W. et al (2013) Neurobio. Dis. 51:72 Tiwari, V. et al (2012) PLOS. DOI: 10.1371/journal.pone.0039451 8.
- 9. Fujisawa, Y. et al (2009) Biochem. J. 423: 219

| Cat. No. | Description | Size |
|--------------|-----------------------------|-------|
| <u>DG049</u> | <u>CHAPS</u> | 1g |
| <u>DG050</u> | <u>CHAPS</u> | 5g |
| <u>DG051</u> | <u>CHAPS</u> | 25g |
| <u>DG096</u> | <u>CHAPS</u> | 100g |
| <u>DG097</u> | CHAPS, 5% filtered solution | 50ml |
| DG098 | CHAPS, 5% filtered solution | 100ml |
| DG099 | CHAPS, 5% filtered solution | 500ml |

CHAPSO

3-[(3-Cholamidopropyl)dimethylammonio]-2hydroxy-1-propanesulfonate

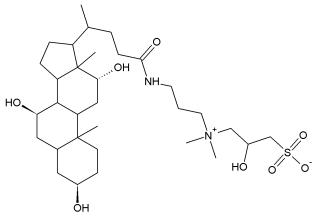


Figure 26: Structure of CHAPSO.

Type: Zwitterionic detergent Mol. Formula: C₃₂H₅₈N₂O₈S Mol. Weight: 630.9 Form: White solid **Purity: >99%** Solubility: Water soluble Conductivity: <50µS in a 10% solution Critical micelle concentration (CMC): 8mM (25°C) Aggregation number: 11 Cloud point: 90°C Average micellar weight: 7000

Application: Zwitterionic detergent. Non-denaturing. Electrically neutral. Higher solubility than CHAPS because of a more polar head group. Solubilizes membrane proteins in their native state. Solubilizes opiate receptor to a state exhibiting reversible binding of opiates

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG052</u> | CHAPSO | 1g |
| <u>DG053</u> | CHAPSO | 5g |

Zwitterionic Detergents

Sulfobetaine 3-10 (SB 3-10)

N-Decyl-N,N-dimethyl-3-ammonio-1propanesulfonate

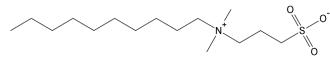


Figure 27: Structure of Sulfobetaine 3-10.

Type: Zwitterionic detergent Mol. Formula: CH₃(CH₂)₉N⁺(CH₃)₂CH₂CH₂CH₂SO₃⁻ Mol. Weight: 307.5 Form: White solid Purity: >99% Solubility: Water soluble Critical micelle concentration (CMC): 25-40mM (25°C) Aggregation number: 41 Average micellar weight: 12,600 Annication: Zwitterionic detergent for colubilization of m

Application: Zwitterionic detergent for solubilization of membrane proteins in their native state

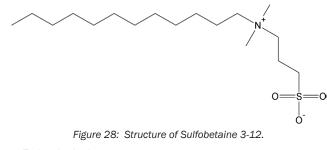
CITED REFERENCES

1. Basic, A. et al (2017) BMC Microbiol.DOI: 10.1186/s12866-017-0967-9

| Cat. No. | Description | Size |
|--------------|-------------------|------|
| <u>DG054</u> | Sulfobetaine 3-10 | 1g |
| <u>DG055</u> | Sulfobetaine 3-10 | 5g |

Sulfobetaine 3-12 (SB 3-12)

N-Dodecyl-N,N-dimethyl-3-ammonio-1propanesulfonate

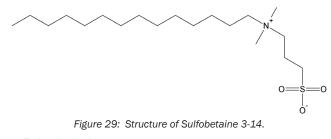


Type: Zwitterionic detergent Mol. Formula: $CH_3(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ Mol. Weight: 335.5 Form: White solid Purity: >99% Solubility: Water soluble Critical micelle concentration (CMC): 2-4mM (25 °C) Aggregation number: 55 Average micellar weight: 18,500 Application: Zwitterionic detergent for solubilization of membrane proteins in their native state

| Cat. No. | Description | Size |
|----------|-------------------|------|
| DG056 | Sulfobetaine 3-12 | 1g |
| DG057 | Sulfobetaine 3-12 | 5g |

Sulfobetaine 3-14 (SB 3-14)

N-Tetradecyl-N,N-dimethyl-3-ammonio-1propanesulfonate



Type: Zwitterionic detergent Mol. Formula: $CH_3(CH_2)_{13}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$ Mol. Weight: 364.0 Form: White solid Purity: >99% Solubility: Water soluble Critical micelle concentration (CMC): 0.1-0.4mM (25 ° C) Aggregation number: 83 Average micellar weight: 30,200 Annication: Zwitterionic determent for colubilization of model

Application: Zwitterionic detergent for solubilization of membrane proteins in their native state

| Cat. No. | Description | Size |
|--------------|-------------------|------|
| <u>DG058</u> | Sulfobetaine 3-14 | 1g |
| DG059 | Sulfobetaine 3-14 | 5g |

ASB-14

Amidosulfobetaine-14

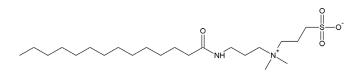


Figure 30: Structure of ASB-14.

Type: Zwitterionic detergent Mol. Formula: $C_{22}H_{46}N_2O_4S$ Mol. Weight: 434.7 Form: White to off white powder Purity: >99% Solubility: Water soluble

Conductivity: <50µS in a 10% solution

Critical micelle concentration (CMC): 8mM (25°C)

Application: Zwitterionic detergent. Aminosulfobetaine with C_{14} alkyl tail. Useful for solubilizing proteins for 2D analysis. Optimal solubility achieved in urea-thiourea mixtures and not in urea alone. Reported to show better protein solubilization properties than CHAPS. ASB-14 has been shown to solubilize membrane proteins previously undetected

| Cat. No. | Description | Size |
|--------------|---------------|------|
| <u>DG060</u> | <u>ASB-14</u> | 1g |
| <u>DG061</u> | <u>ASB-14</u> | 5g |

Amidosulfobetaine-16

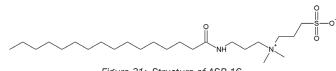


Figure 31: Structure of ASB-16.

Type: Zwitterionic detergent Mol. Formula: $C_{24}H_{50}N_2O_4S$ Mol. Weight: 462.7 Form: White to off white powder Purity: >99%

Solubility: Water soluble

Conductivity: <50µS in a 10% solution

Critical micelle concentration (CMC): 8mM (25°C)

Application: Zwitterionic detergent. Aminosulfobetaine with C_{16} alkyl tail. In some cases superior than ASB-14. Useful for solubilizing proteins for 2D analysis. Optimal solubility achieved in urea-thiourea mixtures and not in urea alone. Reported to show better protein solubilization properties than CHAPS. ASB-16 has been shown to solubilize membrane proteins previously undetected

CITED REFERENCES

McCoin, C. et al (2015) Am J Physiol. DOI: 10.1152/ajpendo.00602.2014
 Shrake, R. et al (2014) J Colloid Interface Sci. 437:140

| Cat. No. | Description | Size |
|----------|---------------|------|
| DG062 | <u>ASB-16</u> | 1g |
| DG063 | ASB-16 | 5g |

ASB-C8Ø

4-n-Octylbenzoylamido-propyl-dimethylammonio sulfobetaine

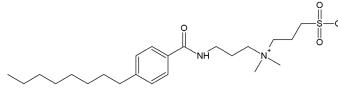


Figure 32: Structure of ASB-C8Ø.

Type: Zwitterionic detergent **Mol. Formula:** $C_{23}H_{40}N_2O_4S$ **Mol. Weight:** 440.6 **Form:** Off white powder **Purity:** >99%

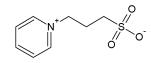
Solubility: Water soluble

Application: A Zwitterionic aminosulfobetaine with an aromatic core that stabilizes and solubilizes integral membrane proteins. Useful for solubilizing proteins for 2D analysis

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG064</u> | ASB-C8Ø | 1g |
| DG065 | ASB-C8Ø | 5g |

NDSB 201

3-(1-Pyridino)-1-propane sulfonate



NON-DETERGENT SULFOBETAINE

Figure 33: Structure of NDSD 201.

Type: Non-detergent sulfobetaine Mol. Formula: $C_8H_{11}NO_3S$ Mol. Weight: 201.4 Form: White powder Purity: >99% Solubility: Water Application: NDSR 201 is a swift/

Application: NDSB 201 is a zwitterionic compound. Unlike zwitterionic detergents, the hydrophobic group in NDSB 201 is too short to form micelles, even at 1M concentrations. NDSB 201 has been used for purification of proteins and solubilization of protein samples for 2D gel electrophoresis

CITED REFERENCES

1. Otsuka, T. et al (2014) Infect. Immun. doi: 10.1128/IAI.01832-14

| Cat. No. | Description | Size |
|--------------|-------------|------|
| <u>DG080</u> | NDSB 201 | 25g |
| DG081 | NDSB 201 | 100g |

Detergent Selection Guide

| Detergent | Туре | Cat. No. | Size | Molecular Weight | Critical Micelle Concentration(mM) | Aggregation No.² | HLB ³ | Average Micellar Weight | Cloud Point (°C) | Conductivity (µS) | Sterile |
|---|---|---|---|---------------------|---------------------------------------|---------------------|------------------|-------------------------------|------------------------|----------------------|---------|
| | | | | NON IONIC | DETERGENTS | | | | | | |
| BigCHAP | Research Grade | DG023 DG024 | 1gm 5gm | 878.1 | 3.4 | 10 | _4 | 8,800 | - | <25 in 10% solution | |
| Deoxy BigCHAP | Research Grade | DG025 DG026 | 1gm 5gm | 862.1 | 1.1-1.4 | 8-16 | - | 10,500 | - | - | |
| | Research Grade | <u>786-521</u> | 500gm | | | | | | | - | |
| Brij® 35 | Proteomic Grade (10% aqueous solution) | DG003 DG004 DG503 DG515 | 5 x 10ml 10 x 10ml 50ml 100ml | 627 | 0.09 | 24-40 | 16.9 | 48,000 | >100 | <50 | Yes |
| Brij® 58 | Research Grade Proteomic Grade (10% aqueous solution) | 786-522 DG005 DG006 DG505 DG516 | 500gm 5 x 10ml 10 x 10ml 50ml 100ml | 1122 | 0.007-0.077 | 70 | 15.7 | 79,000 | >100 | - <50 | Yes |
| MEGA 8 | Research Grade | <u>DG017</u> <u>DG018</u> | 1gm 5gm | 321.4 | 58 | - | - | - | - | - | |
| MEGA 9 | Research Grade | <u>DG019</u> <u>DG020</u> | 1gm 5gm | 335.4 | 19-25 | - | - | - | - | - | |
| MEGA 10 | Research Grade | DG021 DG022 | 1gm 5gm | 349.5 | 6-7 | - | - | - | - | - | |
| Nonidet [®] P-40 Substitute | Research Grade Proteomic Grade (10% aqueous solution) 2D-Detergent [™] (10% aqueous solution) | DG501 DG514 DG901 | 500ml 1 liter 5 x 10ml 10 x 10ml 50ml 100ml 5 x 10ml 10 x 10ml | 573 | 0.05-0.3 | - | - | - | 45-50 | <50 | Yes |
| Octyl ß Glucoside | Research Grade | DG902 DG015 DG016 | 10 x 10m 1gm 5gm | 292.4 | 20-25 | 84 | - | 25,000 | >100 | - | |
| Triton® X-100 | Research Grade Proteomic Grade (10% aqueous solution) 2D-Detergent [™] (10% aqueous solution) | DG507 DG517 DG907 | 500ml 1 liter 5 x 10ml 10 x 10ml 50ml 100ml 5 x 10ml | 647 | 0.2-0.9 | 100-155 | 13.5 | 80,000 | 65 | - <50 <15 | Yes |
| Triton® X-114 | Research Grade Proteomic Grade (10% aqueous solution) | 786-515 786-516 DG009 | 10 x 10ml 500ml 1 liter 5 x 10ml 10 x 10ml 50ml 100ml | 537 | 0.35 | - | 12.4 | - | 23 | - <50 | Yes |
| Tween® 20 | Research Grade Proteomic Grade (10% aqueous solution) | 786-517 786-518 DG011 DG012 DG511 | 500ml 1 liter 5 x 10ml 10 x 10ml 50ml | 1227 | 0.06 | - | 16.7 | - | 76 | - <50 | Yes |
| Tween® 80 | Research Grade Proteomic Grade (10% aqueous solution) | DG519 786-519 786-520 DG013 DG014 DG513 DG520 | 100ml 500ml 1 liter 5 x 10ml 10 x 10ml 50ml 100ml | 1325 | 0.012 | 60 | 15 | 76,000 | 65 | - <50 | Yes |

| Detergent | Туре | Cat. No. | Size | Molecular Weight | Critical Micelle Concentration(mM) | Aggregation No.² | HLB ³ | Average Micellar Weight | Cloud Point (°C) | Conductivity (µS) | Sterile |
|-------------------------|-----------------|----------------------------------|----------------------------|---------------------|---------------------------------------|---------------------|------------------|-------------------------------|------------------------|----------------------|---------|
| IONIC DETERGENTS | | | | | | | | | | | |
| СТАВ | Proteomic Grade | <u>DG094</u> <u>DG095</u> | 25gm 100gm | 364.5 | 1 | 170 | - | 62,000 | - | - | |
| Deoxycholate | Proteomic Grade | <u>DG090</u> <u>DG091</u> | 100gm 500gm | 414.6 | 4-8 | 3-12 | 16 | 1,200- 5,000 | - | - | |
| | Proteomic Grade | DG092 DG093 | 100gm 500gm | | | | | | | | |
| SDS | 10% Solution | <u>R014</u> | 100ml | 288.38 | 7-10 | 62 | 40 | 18,000 | >100 | - | |
| | 20% Solution | <u>786-016</u> <u>786-017</u> | 500ml 1L | | | | | | | | |
| ZWITTERIONIC DETERGENTS | | | | | | | | | | | |
| ASB-14 | Proteomic Grade | <u>DG060</u> <u>DG061</u> | 1gm 5gm | 434.7 | 8 | - | - | - | - | <50 in 10% solution | |
| ASB-16 | Proteomic Grade | DG062 DG063 | 1gm 5gm | 462.7 | 8 | - | - | - | - | <50 in 10% solution | |
| ASB-C8Ø | Proteomic Grade | DG064 DG065 | 1gm 5gm | 440.6 | - | - | - | - | - | - | |
| CHAPS | Proteomic Grade | DG049 DG050 DG051 DG096 | 1gm 5gm 25gm 10gm | 614.9 | 6-10 | 10 | - | 6,150 | >100 | <25 in 10% solution | |
| CHAPSO | Proteomic Grade | DG052 DG053 | 1gm 5gm | 630.9 | 8 | 11 | - | 7,000 | 90 | <50 in 10% solution | |
| Sulfobetaine 3-10 | Proteomic Grade | DG054 DG055 | 1gm 5gm | 307.5 | 25-40 | 41 | - | 12,600 | - | - | |
| Sulfobetaine 3-12 | Proteomic Grade | <u>DG056</u> <u>DG057</u> | 1gm 5gm | 335.5 | 2-4 | 55 | - | 18,500 | - | - | |
| Sulfobetaine 3-14 | Proteomic Grade | <u>DG058</u> <u>DG059</u> | 1gm 5gm | 364 | 0.1-0.4 | 83 | - | 30,200 | - | - | |
| | | | | NON-DETERGE | NT SULFOBETAINE | | | | | | |
| NDSB 201 | Proteomic Grade | <u>DG080</u> <u>DG081</u> | 25gm 100gm | 201.4 | No mic | elles are forme | ed | | - | - | |

¹Critical Micellular Concentration (CMC) determined at 20-25 °C. CMC is the concentration at which micelles begin to form.

 $^2\mbox{Aggregation number}$ is the average number of monomers in a micelle.

³Hydrophile-Lipophile Balance (HLB) defines the hydrophilic character of a detergent.

⁴Data not available

Triton is a registered trademark of Union Carbide Corp; Tween is a registered trademark of Uniqema, a business unit of ICI Americas, Inc.; Nonidet is a registered trademark of Shell Chemicals; Brij is a registered trademark of ICI Americas, Inc.

G-Biosciences offers a range of detergent removal systems that use either a rapid column based system or a precipitation system.

Our products are designed to remove a wide variety of detergents, including SDS, Tween[®] 20, Triton[®] X-100, Triton[®] X-114, Nonidet[®] P-40, CTAB, CHAPS, deoxycholate and Lubrol[®].

Detergent Removal

DetergentOUT[™] GBS10

Detergents are essential for protein solubility during protein extraction and sample preparation, especially when working with hydrophobic proteins. The presence of high concentrations of detergents in protein samples can impair ELISA, IEF, protease digestion of proteins and suppress peptide ionization when analyzed by mass spectrometry.

The resin removes free, unbound anionic, nonionic or zwitterionic detergents (e.g. SDS, Triton® X-100 or CHAPS) from aqueous protein and peptide samples with minimal sample loss for downstream analysis by mass spectrometry and other techniques.

The DetergentOUT[™] GBS10 columns were shown in independent studies to be fully compatible with DI-QTOF and LC-MS/MS (see references). The use of the DetergentOUT[™] GBS10 columns significantly increased the number of peptide spectra detected. In addition, the DetergentOUT[™] GBS10 columns have a high binding capacity for detergents, i.e. 6mg SDS and 14mg Triton® X-100 by every ml settled resin.

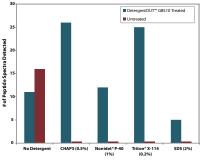


Figure 34: DetergentOUT[™] GBS10 removes detergent & allows detection of peptide fragments by mass spectrometry. 500µg phosphorylase B was digested in solution & then the indicated amount of detergent was added. Samples were treated with DetergentOUT[™] GBS10. Number of peptide spectra were determined as per the protocol of Alvarez, S. et al. A. No detergent, No DetergentOUT[™] GBS10 B. 0.5% CHAPS, DetergentOUT[™] GBS10 treated

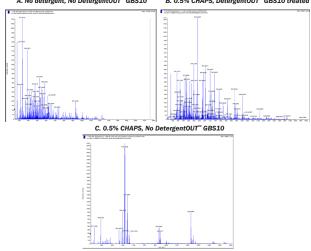
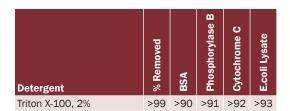


Figure 35: DetergentOUT[™] GBS10 enhances mass spectrometry spectra. 5µg/µl protein mixture (BSA, cyctochrome C and phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B and C). The CHAPS containing sample was treated with DetergentOUT™ GBS10 and compared to an untreated sample (Panel C). Spectra were generated per Alvarez et al.



| Triton X-114, 2% | >96 | >99 | >98 | >97 | >91 |
|-------------------------|-----|-----|-----|-----|-----|
| Nonidet P-40, 1% | >96 | >93 | >95 | >91 | >91 |
| Brij 35, 1% | >99 | >98 | >99 | >97 | >91 |
| SDS, 2.5% | >99 | >96 | >97 | >92 | >90 |
| Sodium deoxycholate, 5% | >99 | >99 | >99 | >98 | >95 |
| CHAPS, 3% | >99 | >92 | >95 | >92 | >91 |
| Octyl glucoside, 5% | >99 | >93 | >95 | >96 | >91 |
| Lauryl maltoside, 1% | >97 | >99 | >99 | >99 | >91 |

Table 1: A comparison of the detergent removal rates and percentage protein recovery with DetergentOUT[™] GBS10.

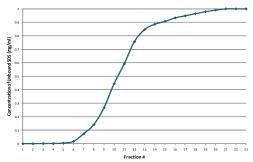


Figure 36: DetergentOUT[™] GBS10 retains ≤6mg SDS per ml settled resin. SDS solution was continuously applied to DetergentOUT[™] GBS10 column. The graph depicts the amount of SDS detected in the flowthrough. SDS was not detected until fraction 7, so after 12mg SDS had been retained by the 2ml of DetergentOUT[™] GB-S10 resin, resulting in a 6mg/ml settled resin binding capacity.

CITED REFERENCES

- Srivastasva, O.P. et al (2017) Biochemistry and Biophysics Report. http://dx.doi.org/10.1016/j. 1. bbrep.2017.01.011
- 2 Min, L. et al (2015) Electrophoresis. DOI: 10.1002/elps.201400579
- З. Valente, K. N. et al (2014) Biotechnol Bioeng. DOI: 10.1002/bit.25515
- 4. Hou, S. et al (2013) Methods, 61:269 5.
- Higgins, D. et al (2005) Anitmicrob. Agents Chemother. 49:1127 6. Hashii, N. et al (2005) Proteomics. 5:4665

| Cat. No. | Description | Sample Size (µl) | Resin (µI) | Size |
|----------------|--|---------------------|---------------|------------|
| <u>786-154</u> | DetergentOUT GBS10-125 | 10-30 | 125 | 10 columns |
| <u>786-155</u> | DetergentOUT [™] GBS10-800 | 30-200 | 800 | 10 columns |
| <u>786-156</u> | DetergentOUT [™] _GBS10-3000 | 200-750 | 3,000 | 10 columns |
| <u>786-157</u> | DetergentOUT [™] GBS10-5000 | 500-1,250 | 5,000 | 10 columns |
| <u>786-998</u> | DetergentOUT [™] GBS10 Spin Plates | 30-200 | 800 | 2 plates |
| <u>786-159</u> | DetergentOUT [™] GBS10 Resin | - | - | 10ml resin |

OrgoSol DetergentOUT[™]

Suitable for hydrophobic proteins, removes detergents and concentrates protein solutions

OrgoSol DetergentOUT[™] is suitable for removal of detergents from protein solutions, including hydrophobic protein solutions and is compatible with all detergent types. Its performance is not dependent on detergent concentration in the solution, is highly flexible and can process small and large sample volumes.

OrgoSol DetergentOUT[™] first concentrates the protein solution through precipitation and then the detergent is extracted and removed with the supplied OrgoSol[™] buffer. The proprietary precipitation agent ensures >99% protein recovery, however precipitation may result in some loss of a protein's biological activity.

Two sizes are offered: Micro Kit for processing up to a total of 10ml protein solution and Medi Kit for processing up to a total of 30ml protein solution, either in a single or multiple experiments.

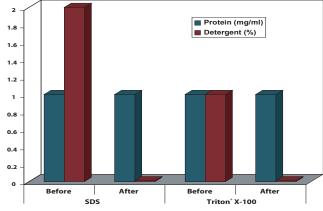


Figure 37: Removal of Detergent. Hydrophobic nuclear fraction proteins (1mg/ml) in 2% SDS and 1% Triton[®] X-100 before and after OrgoSol DetergentOUT[™] treatment.

CITED REFERENCES

- Cortes, D.F. et al (2012) Electrophoresis. 33:3712
 Orr, S.J. et al (012) Molecular Sys Biol. DOI: 10.1038/msb.2012.5
- Orr, S.J. et al (012) Molecular Sys Biol. DOI: 10.1038/msb.20
 Troese, M.J. et al (2011) Infect Immunol. 79:4696

| Cat. No. | Description | Size |
|----------------|---|----------|
| | OrgoSol DetergentOUT [™] , Micro | For 10ml |
| <u>786-128</u> | <u>OrgoSol DetergentOUT[™], Medi</u> | For 30ml |

DetergentOUT[™] Tween[®]

Removal of Tween® (polysorbate) detergents

A spin column format detergent removal resin for polysorbate or Tween[®] detergents or surfactants. DetergentOUT[™] Tween[®] removes polysorbate detergents without significant loss of proteins, dilution of the protein solution, or change to the buffer composition of the protein solution.

| Cat. No. | Description | Size |
|----------------|--|------------|
| <u>786-214</u> | DetergentOUT [™] Tween [®] , Micro | 10 columns |
| <u>786-215</u> | DetergentOUT [™] Tween [®] , Medi | 10 columns |

DETERGENT OPTIMIZATION

Optimizer *blue***BALLS**[™]

Establish optimal detergent conditions

The "Critical Micelle Concentration" (CMC) of a detergent varies with temperature, pH, ionic strength, detergent concentration, purity and presence of organic agents in the detergent. Using a large excess of detergent may pose problems during purification or other downstream applications.

A simple hydrophobic dye solubilization method for the determination of CMC has been described (1) and involves the solubilization of a dye in a detergent solution only in the presence of micelles. The amount of dye solubilized is directly proportional to the micelle concentration. The CMC is determined by plotting optical density of the solubilized dye against detergent concentration. The points of inflection on the plot of observed data versus detergent concentration correspond to the CMC of a typical detergent.

This method is simple and comparable to CMC determined by expensive light scattering or surface tension methods. Furthermore, this method is applicable to all detergents.



Figure 38: Graphical representation of critical micelle concentration determination. Blue colored Optimizer blueBALLS[™] imitate membrane proteins and solubilize when the critical micellar concentration is reached, releasing a non-reactive blue color into the extraction buffer.

FEATURES

- Hydrophobic blue dye coated glass balls that behaves as membrane proteins
- · Add to extraction or perform parallel extractions
- · Ensures optimal detergent concentration is used for extraction
- · Improve downstream processing results
- · Compatible with all detergent types

APPLICATIONS

· Tool for establishing an optimal protein extraction protocol

CITED REFERENCES

- 1. Chang, Y. H. et al (2015) J. Proteome. Res. 14:1587
- 2. Falkinham, J.O. et al (2012) Tuberculosis. 92:173
- Maisuria, B.B. et al (2011) Bioorganic & Medicinal Chemistry 19:2918
 Deutschle, T. et al (2006) Toxicol In Vitro. 20:1472

| Cat. No. | Description | Size |
|----------|---|------|
| DGA01 | <u>Optimizer blueBALLS</u> [™] | 500 |

Detergent Assays

DETERGENT ASSAY

CMC-535[™] Detergent Assay

The CMC-535[™] Detergent Assay is a simple, fluorescent assay designed for the detection of various detergents in aqueous solutions and is an ideal compliment for the DetergentOUT[™] detergent removal columns.

The basic principle of the assay is the interaction of detergents with the CMC-535[™] Fluorescent Dye, resulting in an enhancement of the fluorescent signal that is proportional to the detergent concentration.

The assay can be used to quantitate detergent levels with the use of a standard curves or can be used to compare detergent removal rates to a diluted starting material sample.

The assay is designed to detect detergents at concentrations below their CMC values for most detergents. The assay is compatible with most aqueous buffers, with the exception of buffers that contain phosphates, including molecules that release phosphates (i.e. ADP and ATP). As the assay is sensitive to molecules with strong hydrophobic segments, we recommend <1mg/ml protein and <0.1mg/ml nucleic acids. Suitable for 200 microwell assays.

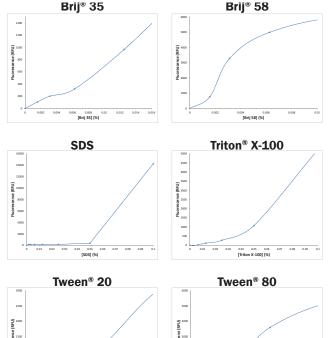


Figure 39: A selection of detergent standard curves produced with CMC-535[™] Detergent Assay.

| Cat. No. | Description | Size |
|--------------|--------------------------------------|----------------------|
| <u>DG535</u> | CMC-535 [™] Detergent Assay | 200 Microwell Assays |

SDS Detection & Estimation

A reagent kit for detection and estimation of SDS in a sample. Mix the test sample in the extraction buffer reagents provided with the kit. If SDS is present in the sample, a blue color is extracted that can be quantitatively measured.

CITED REFERENCES

- Kapoor, M. and Rajagopal, R. (2011) Int. Biodeter. Biodegrad. 65:896 1. 2
 - Sivakumar, S. et al (2007) JBC 282:7312

| Cat. No. | Description | Size | |
|----------------|-------------------|-----------|--|
| <u>786-129</u> | SDS-Detection Kit | 15 assays | |

G-Biosciences Product Line Overview



| | | | СВ-Х | |
|---|--|---|---|---|
| | Estimation | 7 Assays | Non Interfering SPN RED 660 dotMETRIC BCA CB | |
| | | Extraction & Lysis | Sample Grinding Lysis Buffers | Mild Denaturing Strong Chaotropic Specialized |
| | Isolation | Fractionation & Enrichment | 12 Fractionation Kits Dialysis (Micro) | Specializeu |
| | | Sample Preparation | Concentration Contamination Removal | Desalting Detergent Removal |
| | | Reagents | Protease Inhibitors Detergents Chaotropes | General Cocktails Species Specific Individual Inhibitors |
| | | Electrophoresis | 1D & 2D Reagents Gel Stains | 2D Specific Kits Buffers & Reagents Coomassie Silver Reversible |
| | Detection | Western Blotting | 1 Hour System Blocking Agents Secondary Antibodies Chemiluminescence Detection | Non-Animal Animal Non-Protein |
| | | Mass Spectrometry | Trypsin, Mass Spec Grade InGel Kits Coated Plates | |
| | | Assays (ELISA) | Blocking Agents Secondary Antibodies Detection Reagents | Non-Animal Animal Non-Protein |
| | | Affinity Resins | 6X His Tag GST Tag Biotin Tag | Nickel resin Cobalt resin Copper resin Zinc Resin Glutathione Resin Streptavidin Resin |
| | Purification | Activated Resins | CBP Tag Sulfhydryl reactive Amine reactive Carboxyl reactive Drug/ Steroid reactive Protein A or G | Calmodulin Resin |
| | | Antibody Purification | Pearl Resin Biotin | |
| | | Labeling | Fluorescent Dye Enzyme (HRP/AP) | |
| | Modification | Reducing Agents Alkylating Agents | | |
| | | Protein Cleavage Iodination | | |
| | | Amino Acid Side Chain Modifiers | | BSA |
| | | Production | Carrier Proteins Peptide Coupling | KLH HyperCarrier |
| | Antibody | Purification | Protein A or G Resin Activated Resins Pearl Resin Thiophilic Resin Ficin | |
| | | Fragmentation | Pepsin Papain | |
| | SAM Methyltransferase Cell Toxicity & Proliferation | Continuous, Enzymatic Assays Lactate Dehydrogenase (LDH) SRB WST-1 | | |
| ~ | Apoptosis | Caspase | Assays Substrates Inhibitors | |
| | Protease | Assays | | |
| | Phosphatase Peroxide | | | |
| | B-Galactosidase | CPRG Fluorescent (MUG) | | |
| | Genomic DNA | Isolation | Tissue di Blood Plant di Yeast di Bacteria di Fungi di Mouse Tail | |
| | Plasmid DNA | Isolation Colony Screening Transformation | | |
| | Electrophoresis | Apparatus Loading Dyes DNA Ladders Gel Extraction | | |
| | PCR | Taq dNTPs | | |
| | RNA | Extraction RNase Decontamination | ∧D | |
| | Yeast | Transformation Plasmid Isolation | u D | iosciences.com |
| | | | | |

