TECHNICAL BULLETIN #100

How to Purify a Membrane Protein

1

Look up other purifications from similar sources and similar membranes. Different tissues, organisms and membrane types sometimes require different detergents and conditions. e.g. bacterial sources may require quite distinct solubilization procedures from mammalian sources.

2

Complete dispersion and lipid depletion are critical for successful chromatography. If the protein is not separately solubilized from other proteins and much of adhering phospholipid removed, the protein will not bind and chromatograph on the basis of its own characteristics. Instead, it will behave heterogeneously on the basis of the other proteins and lipids attached.

3

A detergent that is good for solubilizing intact membranes and removing excess lipid may not be the best for continuing to purify the “naked” protein (may be too harsh). Thus, for the initial steps, you may want to choose a relatively cheap, relatively pure detergent that can be readily exchanged for another. For example, cholate and CHAPS are both strong, charged or zwitterionic, steroid detergents that break up membranes well and help in removal of excess lipid in chromatographic steps such as hydroxyapatite. They also have a high critical micelle which means they can be readily removed by dialysis or exchanged for another on a column.

4

A good dispersing detergent that will also stabilize the protein and is free of contaminants that could inactivate the enzyme, is needed for chromatographic purification. For many, TRITON® X-100 has been very suitable, but has the problem of contaminants and 280 nm absorbance. A twelve carbon chain length of hydrocarbon tail is often necessary for good stabilization and dispersion, especially for larger multisubunit enzymes. Dodecyl-β-D-maltoside often works equally well or better than TRITON®, with none of the disadvantages. Shorter chain detergents and some charged detergents tend to dissociate multisubunit proteins. Trial and error is clearly important at this stage with careful attention to maintaining a high enough detergent to protein ratio to make sure the protein is well dispersed (the detergent concentration will be much higher than the CMC).

5

Even if a protein is not active in a particular detergent, if you can show that the inactivity is reversible, by adding back lipid or another detergent, you may still be able to use it.

Anatrace

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APX100 ANAPOE®-X-100 (TRITON® X-100)

OXIDANT-FREE
Chromatographically purified to contain less than 20µM of equivalent peroxide.
Supplied in a 10% solution under argon gas.

C316 CHAPS, ANAGRADE®

(3-[(3-Cholamidopropyl)-dimethylammonio]-1-propane sulfonate)
FW 614.9 [75621-03-3] $C_{32}H_{58}N_2O_7S$
CMC (H_2O) ~ 8 mM[1] AGGREGATION NUMBER (H_2O)[2]~10
Each lot of CHAPS is analyzed by HPLC and must be greater than 99% pure.
pH (1% solution) 5-8 Conductance (0.5 M solution) < 50 µS
Percent fluorescence due to a 0.1% detergent solution at 345 nm < 10
Absorbance of a 1% detergent solution: 260 nm < 0.06

D310 n-DODECYL-β-D-MALTOPYRANOSIDE, ANAGRADE®

(n-Dodecyl-β-D-maltoside)
FW 510.6 [69227-93-6] $C_{24}H_{46}O_{11}$
CMC (H_2O) ~ 0.17 mM[3] CMC (0.2MNaCl) ~ 0.12 mM[4]
AGGREGATION NUMBER (H_2O)[4]~78-92
Each lot of dodecyl maltoside is analyzed by HPLC and must be greater than 99% pure.
Percent alpha < 2 (HPLC) Percent dodecanol < 0.005 (HPLC)
pH (1% solution) 5-8 Solubility in water at 0-5°C ≥ 20% Conductance (10% solution) < 40 µS
Percent fluorescence due to a 0.1% detergent solution at 345 nm < 10
Absorbance of a 1% detergent solution: 260 nm < 0.06

S1010 SODIUM CHOLATE, ANAGRADE®

FW 430.6 [361-09-1] $C_{24}H_{39}O_5Na$
CMC (pH 9.0) ~ 9.5mM[5] CMC (pH 7.5) ~ 14mM[6]
Each lot of Sodium cholate is analyzed by HPLC and must be greater than 99% pure.
pH (1% solution) 5-8 Solubility in water at 20°C ≥ 40%
Absorbance of a 1% detergent solution: 260nm < 0.1

T1001 TRITON® X-100

FW avg. 647 [9002-93-1]
CMC ~ 0.010 - 0.016% (w/v)[6-8]
t-Oct-C_6H_4-$(OCH_2CH_2)_x$OH, x = 9-10
Each lot of TRITON® X-100 is analyzed by HPLC and must be greater than 99% pure.[1]
Clear viscous liquid

HLB	13.5	pH (5%, water)	6.0-8.0
Cloud point	65°C	Peroxide	< 0.04%
Color APHA	< 100		

PRECAUTIONS: Store at room temperature. Protect from moisture.
NOTES: 1. This material is purified from an industrial grade detergent. Although it is analyzed to be greater than 99%, it may contain closely related detergents which do not separate under the conditions of the HPLC analysis.

REF. **1**. Hjelmeland, L.M., Nebert, D.W. and Osborne, Jr., J.C., Anal. Biochem., 130, 72-82 (1983). **2**. Womack, M.D., Kendall, D.A. and MacDonald, R.C., Biochem. Biophys. Acta, 733, 210-215 (1983) **3**. VanAken, T., Foxall-VanAken, S., Castleman, S. and Ferguson-Miller, S., Methods Enzymol., 125, 27-35 (1986). **4.** Anatrace measurement **5**. Brito, R.M. MandVaz, W.L.C., Anal. Biochem, 152, 250-255 (1986). **6**. Vendittis, E., Paumbo, G., Parlata, G., and Borchini, U., Anal. Biochem., 115, 278-286 (1981) **7**. Ross, S. and Oliver, J.P., J. Phys. Chem., 63, 1671-1674 (1959). **8**. Mankovich, A.M., J. Amer. Oil Chem. Soc., 41, 449-452 (1964)