Screening Detergents for Optimal Solubilization and Purification of Membrane Proteins

Overview

The hydrophobic domain of membrane proteins makes them difficult to solubilize from their anchor in cell membranes. Detergents are amphipathic molecules with a polar head and a long hydrophobic carbon chain that form micelles in which membrane proteins embed and thus, can remain in aqueous solution. The resulting protein-detergent complex can then be isolated and purified.

The selection of the right detergent is crucial for the effective purification of a membrane protein. Factors to bear in mind are: (1) the critical micelle concentration (CMC) of a detergent, or the concentration at which the detergent forms micelles and accumulates in the membrane; (2) that changing protein or salt concentration can change the aggregation behavior of a protein and the CMC of a detergent; (3) that prolonged exposure to a detergent can delipidate a protein which leads to instability; and (4) that certain detergents are suboptimal for subsequent chromatographic purification; for example, ion exchange chromatography does not work well with charged detergents.

This protocol delineates the screening of detergents to solubilize a membrane protein expressed in a bacterial host (see Protocol "Screening for Optimal Membrane Protein Expression Conditions Using E. coli Expression Systems"). The detergent concentrations provided for the solubilization and for buffers used in the purification protocol (Table 1) are a starting point and may need to be adjusted for the target protein. Likewise, parameters such as temperature and length of solubilization can also be optimized in this screen (Fig. 1). Results from the screen are analyzed by Western blot, using a detection antibody specific to the target protein or the fused tag (e.g., His, GST, Strep®-tag, rho-1D4). The best detergent and solubilizing conditions, as determined with this screen, are then implemented for the purification protocol corresponding to the used affinity tag (www.cube-biotech.com/protocols).

Equipment

- UV/VIS Spectrophotometer
- SDS-PAGE and Western blotting equipment
- pH-meter
- Ultrasonic homogenizer
- Waterbath
- Refrigerated tabletop centrifuge
- End-over-end rotator
- 2mL microcentrifuge tubes

Materials

- Tris base
- Sodium chloride (NaCl)
- Glycerol
- Imidazole
- Hydrochloric acid (HCl)
- Sodium dodecyl sulfate (SDS)
- Bromophenol blue
- Dithiothreitol (DTT)
- 1 g pellet of expression host (eg., E. coli) that has completed a round of protein expression under optimized conditions (see Protocol "Screening for Optimal Membrane Protein Expression Conditions using E. coli Expression Systems").
- Lysozyme
- Benzonase® Nuclease (Novagen #72106-3)
- Octyl-β-glucopyranoside (OG, 1 g; Cube BIotech #16001)
- n-Octyl-1-thio-β-D-glucopyranoside (OTG, 1 g; Cube Biotech #16017)
- n-Decyl-β-maltopyranoside (DM, 1 g; Cube Biotech #16009)
- n-Dodecyl-β-maltoside (DDM, 1 g; Cube Biotech #16013)
- FOS-choline®-12 (FC-12)
- N,N-Dimethyldodecylamine-N-oxide (LDAO, 1 g; Cube Biotech #16005)
- A detection antibody that binds to the target protein or protein tag (for Western blot analysis).
- Lysis buffer corresponding to used affinty tag.

Solutions and buffers

Lysis Buffer, 1000 mL

The lysis buffer to use depends on the target protein and the affinity tag. Cube Biotech provides in each tag-specific purification protocol the recipe for a Lysis Buffer that has been tested with the corresponding tag (e.g., His, GST, Strep®-tag, rho-1D4; cube-biotech.com/protocols). This Lysis Buffer should be used as an initial buffer that can be adapted as needed for the target protein.

5X SDS-PAGE Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for final buffer
Tris-HCl, pH 6.8–7.0	300 mM	121.14	1 M	121.14 g/1 L	3 mL
Glycerol	50% (v/v)	—	100%	—	5 mL
SDS	5 % (w/v)	—	—	—	0.5 g
Bromophenol blue	0.05% (v/v)	—	4%	—	125 µL
DTT	250 mM	154.25	1 M	1.54 g/10 mL	125 µL/aliquot

Instructions: Make sure to prepare a 1M Tris-HCl stock by dissolving Tris base in 500 mL deionized water, adding HCl to a pH of 6.8–7.0, and adding water to a final volume of 1 L. For the SDS-PAGE Buffer, mix all components listed **except DDT** and add water to a total of 10 mL. Freeze 20 aliquots (0.5 mL each) at –20°C. Before use, add DTT to the needed single aliquots.

Procedure

1. Using the expression culture scaled up as the last step in Protocol "Screening for Optimal Membrane Protein Expression Conditions using E coli Expression Systems", harvest a 1 g cell pellet by centrifuging at 4,000 rpm and 4°C for 20 min.

   Note: The steps described are for a 1 g pellet. If your pellet is larger or smaller, adjust the amount of buffer, lysozyme and Benzonase correspondingly: 10 mL Lysis Buffer per 1 g cells; 1 mg lysozyme per mL buffer; 12 µL Benzonase per 10 mL buffer (steps 2–4).

2. Complete the Lysis Buffer corresponding to the affinity tag used by adding 1 mg lysozyme per mL buffer needed.

3. Carefully pour off the supernatant from the cell pellet and resuspend in lysozyme-Lysis Buffer. Use 10 mL buffer per g cell pellet.

4. Add 12 µL Benzonase® Nuclease per 10 mL Lysis Buffer, and incubate the resuspended cells for 30 min at room temperature (15–25°C).

   Note: If your protein is particularly sensitive, incubate on ice.

5. Disrupt cells by sonicating in short bursts for 3 min. Repeat once.

   Tip: Keep the lysate on ice to prevent warming.

6. Centrifuge the lysate at 900xg and 4°C for 15 min to remove cell debris.

7. Transfer the supernatant to a fresh tube and centrifuge at 7,000xg and 4°C for 30 min to precipitate inclusion bodies.

8. Transfer the supernatant to a fresh tube, mix briefly, and distribute evenly among the number of 2 mL microcentrifuge tubes needed to test the desired detergents and solubilization conditions.

   These are the screening samples.
   Note: The number of microcentrifuge tubes to use depends on the number of detergents and solubilization conditions to be tested (Fig. 1 shows a generic screen setup).

9. Centrifuge the screening samples at 20,000xg and 4°C for 1 h.

   Note: At this speed only a portion of the membrane pellets out, but this is sufficient for the detergent screen.

10. Discard the supernatants. Label each tube with the detergent and solubilization conditions to be test (e.g., see Fig. 1).

11. Resuspend each pellet in 500 µL Lysis Buffer.

12. Add detergent to each tube in the amount to be tested. The solubilization concentrations given in Table 1 serve as a starting point, but different concentrations should be screened to optimize the solubilization of the target protein.

13. Incubate the samples on an end-over-end rotator. As a starting point, incubate for 1 h at room temperature (15–25°C). However, other incubation times and temperatures should be screened to optimize the solubilization conditions (e.g., Fig. 1).

14. Remove a 20 µL aliquot from each tube for later analysis. Store the aliquots in 5 µL 5X SDS-PAGE Buffer at –20°C.

These are the total protein in each screening sample.

15. Centrifuge the screening samples at 20,000xg and 4°C for 1 h.

16. Remove a 20 µL aliquot from each tube for later analysis. Store the aliquots in 5 µL 5X SDS-PAGE Buffer at –20°C.

These are the soluble membrane fractions of the screening samples.

17. Thaw all aliquots taken for analysis and heat for 30 min at 46°C.

18. Analyze the aliquots by SDS-PAGE and Western blot to identify the detergent and conditions that afforded the best solubilization. Use this detergent and incubation parameters for the purification of the target protein.

Samples from good solubilizing detergents will show a strong band at the corresponding protein size (see Fig.2).

Detergent	Molecular weight (g/mol)	CMC in H_2O (mM)	Solubilization concentration	Amount in 500 µL Lysis Buffer	Concentration for purification*
OG	292.37	~18–20[2]	51 mM	7.46 mg	27–40 mM
LDAO	229.40	~1–2[3]	30 mM	3.44 mg	1.5–4 mM
DM	482.56	~1.6[4]	21 mM	5.07 mg	2.4–3.2 mM
DDM	510.62	~0.17[5]	20 mM	5.11 mg	0.26–0.34 mM
OTG	308.44	~9[6]	51 mM	7.87 mg	13.5–18 mM
FC-12	351.50	~1.5[7]	32 mM	5.62 mg	2.3–3 mM

* Concentration of detergent to be used in the Elution Buffer and the Equilibration and Wash Buffer of the purification protocol.

Table 1: Overview of available Cube Ultrapure Detergents with corresponding CMC, solubilization concentration, and concentration for the purification. The solubilization and purification concentrations are given as a starting point and should be optimized for each target protein. Other detergents can also be screened in a similar fashion. CMC: critical micellar concentration.

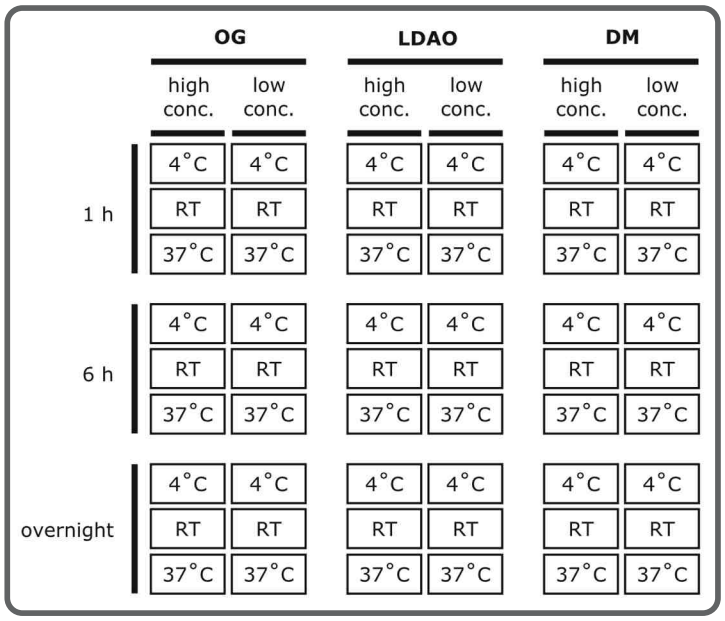

Fig. 1: Generic screen setup for solubilization detergent, concentration, temperature, and time. 3 detergents, 2 concentrations, 3 temperatures, and 3 incubation times are screened with 54 screening samples to determine optimal solubilization conditions. RT: room temperature.

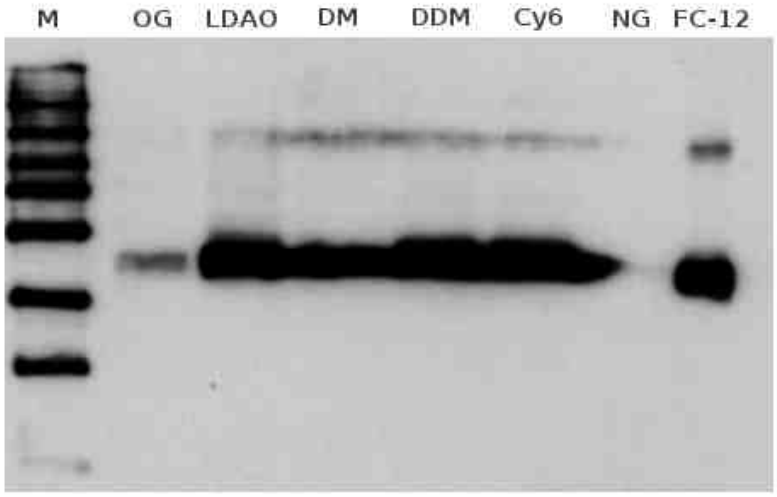

Fig. 2: Western blot analysis of an example screening of 7 detergents. Aliquots of membrane factions solubilized with 7 different detergents were analyzed via SDS-PAGE and Western blot using an antibody specific to the tag fused to the target protein. Detergents OG and NG are poor solubilizers for this protein.

References:

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