Solubilization of membrane proteins using the synthetic polymer diisobutylene-maleic acid (DIBMA)

Procedure

1. With DIBMA you can solubilize membrane proteins in
   a. Suspensions of whole cells
   b. supernatant 9,000
   c. Homogenized 100,000 Pellet
2. You can add directly the isolated membrane protein solution (40 mg/ml (w/v)) (NOTE: pH is already adjusted with TRIS or HEPES) to your DIBMA/Buffer powder to a final concentration of 0,4 % - 4 %. We advise you to add 2 ml of your solution to 50 mg lyophilized DIBMA to start with a DIBMA concentration of 2,5 %. For screening purposes you can try different DIBMA concentrations to find optimized conditions for solubilization.

DIBMA	4 %	3,5 %	3 %	2,5 %	2 %	1,5 %	1 %	0,8 %	0,6 %	0,4 %
Protein solution	1,25 ml	1,4 ml	1,7 ml	2 ml	2,5 ml	3,33 ml	5 ml	6,25 ml	8,33 ml	12,5 ml

4. Optional: It is possible to screen the total protein concentration (beginning from 40 mg/ml) against 2,5 % DIBMA. The DIBMA or protein concentration screening can influence the success of the solubilization of your target protein.
5. With gentle shaking overnight at 4 °C and/or room temperature you can solubilize your proteins. The optimal conditions are protein specific (screen!).
6. The contained TRIS/HEPES buffer keeps the pH at 7.5 which stabilize the functionality of DIBMA and is suitable for the most applications. If you need a different pH or buffer for your protein solubilization we provide DIBMA without buffer. Please note that a pH smaller than 6.5 is not suitable for solubilization with DIBMA.

SDS-PAGE Protocol:

If you want to run a SDS Page your proteins have to be separated from the synthetic polymers. The presence of the polymers can lead to smearing bands on SDS-PAGE gels (1,2). Wessel and Flügge discribed the protocol in 1984 originally (3). It was modified in 2017 for the use with DIBMA (1).

1. Measure volume of solubilized protein - polymer sample
2. Vortex the sample with 4x volume of cold methanol
3. Vortex with 1x volume of chloroform
4. Centrifuge for 3 min at 4 °C and 15,000 g with 3x volume of cold water
5. Add 4x volume of methanol to the organic layer and discard the aqueous.
6. Centrifuge at 5,000 g for 1 min to pellet the proteins.
7. Centrifuge at 20,000 g for 5 min at 4 °C.

References:
1. Oluwole, Abraham Olusegun, et al. „Solubilization of Membrane Proteins into Functional Lipid☐Bilayer Nanodiscs Using a Diisobutylene/Maleic Acid Copolymer.“ Angewandte Chemie International Edition 56.7 (2017): 1919-1924.

2. Lee, Sarah C., et al. „A method for detergent-free isolation of membrane proteins in their local lipid environment.“ Nature protocols 11.7 (2016): 1149.

3. Wessel, D. M., and U. I. Flügge. „A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids.“ Analytical biochemistry 138.1 (1984): 141-143.