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

Procedure

1. As a first step you have to resuspendend your isolated membranes in buffer.
2. Now you can add the lyophilized DIBMA/Buffer powder to a final concentration of 1 % - 3 %.
3. With gentle shaking overnight at 4 °C and/or room temperature you can solubilize your proteins. The optimal conditions for your protein have to be determined by yourself.
4. The contained TRIS/HEPES buffer keeps the pH at 7.5 which is suitable for the most applications. If you need a different pH for your protein solubilization we provide DIBMA without buffer. Please note that a pH smaller then 6.5 is not suitable for solubilization with DIBMA.
5. For protein purification and analysis is no additional DIBMA needed.
6. Because this solubilization is detergent-free you can perform all commonly used protein purification and analysis techniques like ELISA, IMAC, affinity, SEC, UV/Vis spectroscopy, western blotting and SDS-PAGE.

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.
8. Remove supernatant
9. Dry pellet
10. Now you can run SDS-PAGE

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.