Cube Biotech

# Solubilization of membrane proteins using the synthetic polymer styrene-maleic acid (SMA)

## Protocol

1. SMA allows for reconstitution of membrane proteins during various steps of purification.
   It is used with:
   a. whole cell suspensions
   b. supernatant of centrifuged cell lysate (9,000 x g)
   c. pellets of centrifuged supernatant (100,000 x g)

Note: Besides 50 mg SMA the lyophilisate contains Buffer adjusted to pH 7.5 for stabilization.

2. Add the protein solution directly to the ready-to-use lyophilized SMA powder and invert/vortex the tube until the SMA is completely dissolved. It is advised to use 2 mL of your protein solution with 50 mg lyophilized SMA to get a final concentration of 2,5 %. To further optimize your solubilization different SMA concentrations within the range of 0,4 % - 4 % are recommended (Tab. 1).

Table 1: Volume of the protein sample to adjust a 50 mg SMA sample to final concentrations recommended for screening purposes.

| **Volume [mL]** | 12,50 | 7,14 | 5,00 | 3,33 | 2,50 | 2,00 | 1,67 | 1,43 | 1,25 |
|---|---|---|---|---|---|---|---|---|---|
| **SMA (w/v)** | 0,4 % | 0,7 % | 1,0 % | 1,5 % | 2,0 % | 2,5 % | 3,0 % | 3,5 % | 4,0 % |

3. Incubate the mixture at cold temperatures overnight (16 h, 4 °C) while gently stirring/shaking the solution.

4. Collect a sample for Western Blot analysis. Separate solubilized protein from insolubilized protein and debris with a centrifugation step (100 000 x g, 1 h, 4 °C). Draw a sample of the supernatant to validate the solubilisation through Western Blot analysis and save the rest for further use.

5. Note: Protein quantification using UV absorbance spectrophotometry is hindered when using SMA. Take that into consideration.