

SMA™ Polymers

solubilization of membrane proteins using SMA™ Polymers

SMA Polymers are supplied as a stock solution that can be stored at 4°C for 6 months prior to opening. After opening the solution is stable for at least 1 month at 4°C. Below is a generically applicable protocol for solubilization. You may need to modify aspects of the protocol to suit your individual protein.

other resources

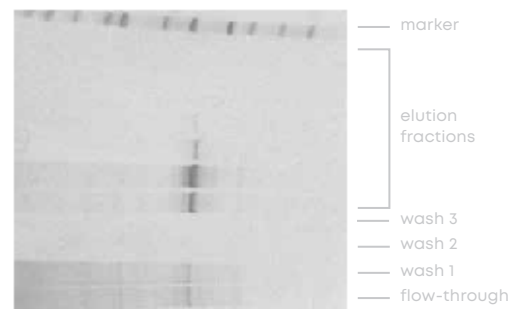
Protocol modified from Lee et al. ⁽¹⁾

starting with cell pellets

1. Prepare membranes from your cells of choice using methods outlined in the biochemical literature (e.g. *Escherichia coli*, *Saccharomyces cerevisiae*, CHO, *Pichia pastoris*)⁽¹⁾.
2. Resuspend the membranes using a homogenizer to produce a final concentration of 40 mg/ml in 500 mM TrisHCl, 500 mM NaCl and 10% Glycerol. Check that the pH of the sample has not moved out of the pH range for the relevant polymer.
3. Allow the SMA Copolymers to equilibrate at room temperature
4. Add SMA Copolymers to the membranes to a concentration of 2.5% wt/vol
5. Incubate the sample for 2 hours at room temperature with gentle stirring. For some membranes you may need to incubate at either higher temperatures (e.g. 37°C for 30 mins) or lower temperatures (e.g. 4°C overnight).
6. Remove insoluble material by centrifugation at 100,000 x g for 45 minutes at 4°C
7. Remove the supernatant (S) and put on ice and reserve the pellet (P)
8. Take 10 µl of the supernatant and 10 µl of the pellet and dissolve in SDS PAGE loading buffer according to relevant manufacturer's instructions
9. Run samples on an SDS PAGE and visualize the protein using Instant Blue Coomassie and assess purity
10. If required establish the presence of the protein in the samples using a Western Blot
11. If protein is present in the supernatant apply to relevant affinity resin and purify
12. Confirm purity using SDS PAGE as above

SDS PAGE showing the Ni-NTA purification of *Escherichia coli* His6-ZipA protein extracted from membranes using SMA Copolymers

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(1) Lee et al. Nat Protoc. 2016 Jul;11 (7) : 1149-62.

The provided protocol within this document acts only as a guide and has no guarantee of success. The exact conditions that can be used can vary depending on the system.

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