Ni-NTA purification

purification of membrane proteins solubilized using SMA™ Polymers

Incorporation of membrane proteins into SMA Based Particles can reduce the a affinity of Histidine tags for immobilized metal a affinity chromatography resins like Ni-NTA. To address this the use of a modified purification protocol is advised.

other resources

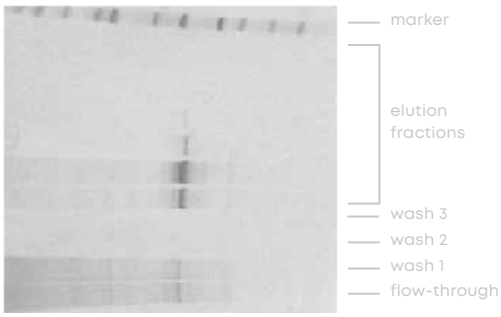
Protocol modified from Lee et al. [1]

starting with SMA Solubilized Protein (see solubilization quick protocol)

1. Wash 1 ml of IMAC resin into 500 mM TrisHCl, 500 mM NaCl and 10% Glycerol by pelleting the resin using centrifugation, discarding the supernatant before resuspending the TrisHCl/NaCl buffer
2. Add the supernatant from the polymer extraction to the resin and leave overnight at 4°C with gentle agitation (by slow inversion)
3. Load the resin into a disposable column
4. Collect the flow through and label “Flow through”
5. Wash resin with 10 column volumes of the TrisHCl/NaCl
6. 6. Collect 1 ml fractions and label (Wash 1-10)
7. 7. Wash with 10 column volumes of 500 mM TrisHCl, 500 mM NaCl, 10% Glycerol, 500 mM Imidazole.
8. Collect 1 ml fractions and label (Elution1-10)
9. Take 10 µl of each fraction and add to SDS loading buffer
10. Run samples on an SDS PAGE and visualize the protein using Instant Blue Coomassie and assess purity
11. If required establish the presence of the protein in the samples using a Western Blot

SDS PAGE showing the NiNTA purification of E. coli His6-ZipA protein extracted from membranes using SMA. NB. The Coomassie Stained smear observed in the second wash is from residual SMI polymer in the preparation

Reproduced with permission from Ms S. Nestorow, University of Birmingham



(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.

Revised 13-MARCH-24

aurorium

201 North Illinois Street, Suite 1800
Indianapolis, IN 46204 USA
ask@aurorium.com
www.aurorium.com