Depletion Of Free Nanodisc Copolymer Using PolyHunter Agarose Resin

When to apply this protocol:

After the copolymer has solubilized the membrane proteins from the

- cell membrane or
- cell lysate or
- whole cells and has formed stable nanodiscs.

And a centrifugation step to to retain only the soluble fractions (stable nanodiscs) has been performed

Before the subsequent affinity chromatography

The protocol:

1. Use 2ml PolyHunter agarose suspension (50%) per 0.5 ml cell lysate that contains 2.5% copolymer used in HEPES (50 mM)
2. Fill PolyHunter agarose into a gravity-flow column (13 mm diameter recommended)
3. Wash agarose 3x with distilled water and equilibrate two times with the same buffer as present in your cell lysate (e.g. 50 mM HEPES, 150 mM NaCl, pH 7.5)
4. Add your solubilizate (0.5 ml) on top of the PolyHunter agarose bed and let the solution penetrate the agarose bed by gravity flow.
5. Discard the flow-through. *This is only the buffer, not your sample.*
6. Place a tube for collection of nanodisc containing fraction under the gravity-flow column
7. Add 1 ml cell lysate buffer (e.g. 50 mM HEPES, 150 mM NaCl, pH 7.5) for elution and collect the eluate

8. **Final step:** The flow-through now contains your protein-nanodisc complex. Do NOT discard this flow- through! This is your final sample!

9. ***Optional:*** The agarose can be regenerated by two times distilled water, Followed by 5x washing with 50 vol% aqueous methanol solution and 5x washing with the cell lysate buffer. In total, the agarose can be reused 5x after thoroughly regeneration.
   Store the beads in 20 mM Sodium acetate, 20% Ethanol, pH 6.5

Side note:

This protocol can be scaled up proportionally. The amounts and volumes mentioned above are therefore only examples.