Depletion Of Free Nanodisc Copolymer Using PolyHunter MagBeads

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:

Use 4ml PolyHunter MagBead suspension (25%) per 2 ml solubilizate that contains 2.5% copolymer used in HEPES (50 mM). *This can be scaled up proportionally.*

1. Fill 4 ml PolyHunter MagBead suspension into a 15 ml tube.
2. Place tube into a MagBeads separator
3. Wash MagBeads 3x with distilled water and two times with the same buffer as present in your cell lysate (e.g. 50 mM HEPES, 150 mM NaCl, pH 7.5). Remove liquid.
4. Add your solubilizate (2 ml) on top of the PolyHunter MagBeads
5. Place tube on a shaker to mix MagBeads and solubilizate. (30 min.)
6. Place tube back in the MagBead separator and collect the polymer depleted cell lysate. **Do not discard it! This is your sample!**
7. Repeat step 3-6 two times for efficient depletion of excess polymer

8. **Final step:** The solubilizate after the last depletion step contains your copolymer depleted protein-nanodisc complex.

9. ***Optional:*** The MagBeads 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.