Protocol

Pretreatment of MagStrep “type3” XT beads

Work at room temperature (max. 25 °C).

1. **Determine how many magnetic beads are needed to purify the target protein.**
   20 µl of the homogenously suspended 5% (v/v) magnetic bead suspension correspond to 1 µl magnetic beads.
   Use 1 µl magnetic beads per 0,85 nmol recombinant protein in the cleared lysate. Cleared lysate should not exceed 2.5 ml per µl beads and, therefore, concentration of recombinant protein should be >10 µg/ml. Higher target protein concentrations are preferable.

   Table: Determination of the protein concentration (1 Da = 1 g/mol)

Size of recombinant protein [kDa]	Measured protein concentration [µg]	Calculated protein concentration [nmol]	Required amount of magnetic beads (5% suspension) [µl]
30	30	1	24
	1500	50	1180
85	335	3,94	93

2. **Pipette the required amount of beads into a vial, place it on the magnetic separator to separate beads and remove the supernatant.**
3. **Equilibrate beads (repeat this step 2 times).**
   - Resuspend beads in 0.2 ml Buffer W per µl beads.
   - Separate beads in the magnetic separator and remove supernatant.
4. **Remove tube from magnetic separator.**
5. **Beads are now ready to use.**

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Protocol (continued)

Purification of recombinant Strep-tag®II or Twin-Strep-tag® fusion protein using MagStrep "type3" XT beads

Immediately prior to affinity purification, centrifuge the extract for 20 min at 10,000 x g (or 4,000 x g for small volumes) to remove any cell debris or aggregated protein.

1. **Resuspend the magnetic beads with the appropriate volume of the cleared extract containing the target protein**
2. **Incubate 30 minutes on ice (vortex occasionally (3-4x) during incubation bringing beads into suspension).**
3. **Place reaction tube in magnetic separator and carefully remove the supernatant.**
4. **Remove the magnet.**
5. **Wash step (repeat this step 3 times):**

   Fast washing will improve target protein yields.
   Continue as follows:
   - Add 100 µl Buffer W per µl beads.
   - Vortex shortly.
   - Quickly place reaction tube in magnetic separator to collect the beads.
   - Remove supernatant.

6. **Elution**

6.1 Elution under native conditions

Elution of bound protein (repeat this step once for higher recovery):

- Remove reaction tube from magnetic separator and add 25 µl Buffer BXT per µl beads and vortex.
- Incubate 10 minutes under occasional vortexing (2-3x) to bring the beads into suspension.
- Place reaction tube in magnetic separator to separate beads.
- Pipet off supernatant containing recombinant protein of interest and transfer it into a clean reaction tube.

The first elution step will yield the target protein at the highest concentration. Pool supernatants from both elution steps to maximize the yield (but not the concentration) and analyze purity via SDS-PAGE and Coomassie or silver staining and quantify according to Bradford using BSA as standard.

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