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Protein Production &
Assays

Strep-tag® Purification using MagStrep “type3” XT Beads

Manual

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Content

1 Introduction

The Strep-tag®II peptide (8 amino acids, WSHPQFEK) binds with high selectivity to Strep-Tactin® and Strep-Tactin®XT – both engineered streptavidins with improved binding properties. A variant of Strep-tag®II is the Twin-Strep-tag® including the motif two times in series connected by a linker. The development of the Strep-tag® system and its applications are reviewed by Schmidt *et al*., 2013.

The latest improvement of the system was the development of Strep-Tactin®XT. It binds the Strep-tag®II with a binding affinity in the low nM range and the Twin-Strep-tag® in the low pM range. When using Strep-Tactin®XT, specific elution has, however, to be performed with 50 mM biotin instead of 2.5 mM desthiobiotin.

Strep-tag®II or Twin-Strep-tag® can be genetically fused upstream or downstream to any gene and expressed as fusion peptide. The technology allows one-step purification of almost any recombinant protein under physiological conditions. But also more stringent conditions like elevated ionic strength, reducing conditions, detergents, metal ions and chelating conditions are possible. pH should be slightly alkaline, best pH 8.

There are almost no restrictions regarding the expression host as many including baculovirus, plants, mammalian cells, yeast, and bacteria have been used successfully yet. Detailed information incl. reviews and scientific publications giving an overview on the Strep-tag® technology are available at www.iba-lifesciences.com.

MagStrep "type3" XT beads are coated with Strep-Tactin®XT. The beads have a high binding capacity (up to 0.85 nmol/µl beads, corresponds to 25.5 µg of a 30 kDa protein) combined with very low non-specific protein binding. If the isolated proteins are not needed in functional form, elution can also be performed by boiling in denaturing SDS gel loading buffer instead of using biotin.

2 Protocol

Required Products/Buffers	Concentration of ingredients	Cat.no.
MagStrep “type3” XT beads	5% (v/v) suspension (PBS, 0.1% (w/v) sodium azide)	2-4090-002 or 2-4090-010
Buffer W (10x)*; Strep-Tactin®/XT Wash Buffer	1 M Tris/HCl, pH 8.0 1.5 M NaCl 10 mM EDTA	2-1003-100
Buffer BXT (10x)*; Strep-Tactin®XT Elution Buffer	1 M Tris/HCl, pH 8.0 1.5 M NaCl 10 mM EDTA 0.5 M Biotin	2-1042-025
Magnetic Separator for 24 reaction tubes		2-1602-000

*has to be diluted 1:9 with H_20 prior to use

General Remarks

- MagStrep “type3” XT magnetic beads are ferrimagnetic spheres coated with 6% cross-linked agarose to which Strep-Tactin®XT is coupled. The average particle diameter is 25 µm. Due to the comparatively large size, high water content and their ability to bind biomolecules within the agarose matrix, magnetic beads are quantified according to the wet volume that they occupy when settled, and not according to their dry weight. Thus, MagStrep “type3” XT magnetic beads are offered as 5% (v/v) suspension, which means that 20 µl of such a homogenous suspension contains an amount of beads that occupies a wet volume of 1 µl. This is the amount to be used if referred to in this protocol.
- The volume of the cleared extract should not exceed 2.5 ml per µl MagStrep ”type3” XT beads and the concentration of the Strep-tag® fusion protein should be >1 mg/ml. Higher concentrations are generally beneficial to increase purification yields according to this protocol. Use 1 µl MagStrep ”type3” XT beads (= 20 µl of the supplied 5% v/v suspension) per nmol target protein to be purified from the extract. If the target protein concentration in the cleared extract is < 1 mg/ml, we highly recommend using our Strep-Tactin®/XT gravity flow purification columns.
- In some cases it may be advantageous to use 100 mM biotin to get the target protein eluted at higher concentration.

Important notes

- In general, Twin-Strep-tag® leads to better yields in batch purification due to its higher binding affinity for Strep-Tactin®XT.
- MagStrep “type3” XT beads are **not** reusable.

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 >1 mg/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 amount [µg]	Calculated protein amount [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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Protocol (continued)

6.2 Elution under denaturing conditions

As an alternative to the elution under native conditions with biotin, the recombinant Strep-tag® fusion protein may also be eluted under denaturing conditions by boiling. To this end, a conventional SDS gel loading buffer is used in the previous step (instead of Buffer BXT) and the sample is heated to 95 °C for 2 min.

Immobilized tetrameric Strep-Tactin®XT will denature under these conditions as well, leading to an additional band at 13.5 kDa during SDS-PAGE analysis. Strep-Tactin®XT magnetic beads exhibit very low non-specific protein binding activity so that no substantial amounts of other contaminating proteins should be detectable.

Schmidt, T.G.M., Batz, L., Bonet, L., Carl, U., Holzapfel, G., Kiem, K., Matulewicz, K., Niermeier, D., Schuchardt, I., and Stanar, K. (2013) Development of the Twin-Strep-tag and its application for purification of recombinant proteins from cell culture supernatants. *Protein Expression and Purification* 92, 54–61.

Please refer to **www.iba-lifesciences.com** for further information regarding “Reagents compatible with Strep-tag®/Strep-Tactin®XT interaction”.

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