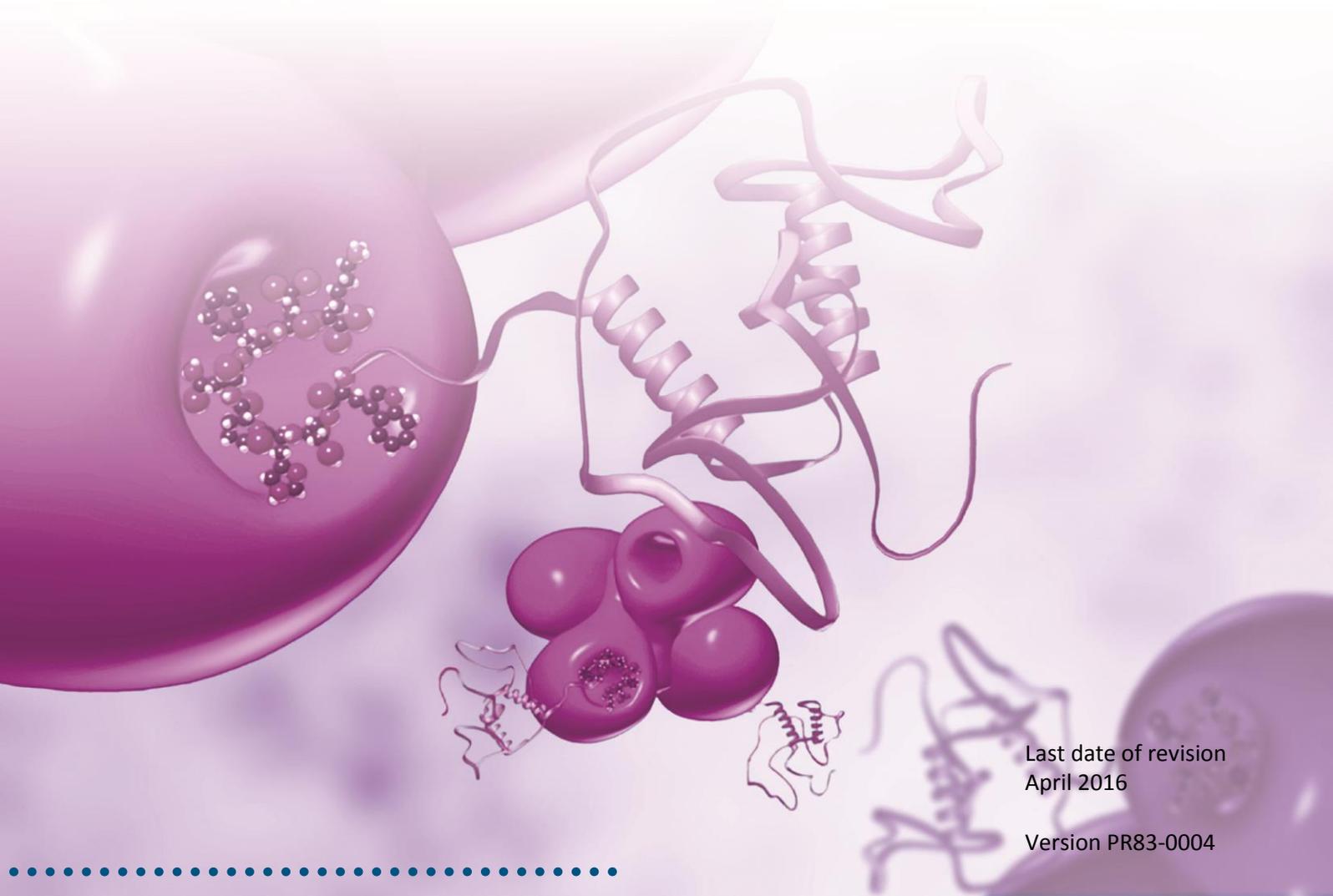


Strep-tag[®] Purification using MagStrep “type3” XT Beads

Instruction manual



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For research only

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This manual can be downloaded under www.iba-lifesciences.com/technical-support.html.

1 Introduction

The Strep-tag®II peptide (8 amino acids, WSH₂PQFEK) binds with high selectivity to Strep-Tactin® - an engineered streptavidin with improved binding properties. A variant with further increased affinity under preservation of reversibility is its sequential arrangement called Twin-Strep-tag (28 amino acids, WSH₂PQFEKGGGSGGGSGGSAWSHPQFEK). These developments including several applications are reviewed by Schmidt *et al.*, 2013. The latest improvement was the development of Strep-Tactin®XT, which binds the Strep-tag®II by 1-2 orders of magnitude and the Twin-Strep-tag by 3-4 orders of magnitude tighter than regular Strep-Tactin® – still under preservation of reversibility (Schmidt *et al.*, to be published). When using Strep-Tactin®XT, specific elution has, however, to be performed with 10 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. This 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, however, be > 7, preferably 8. There are also 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 over Strep-tag® technology are available at www.iba-lifesciences.com.

MagStrep “type3” XT beads are coated with Strep-Tactin®XT. The beads have high binding capacity (~1 nmol/μl beads, corresponding to 30-90 μg of a 30 kDa protein) combined with very low non-specific protein binding. Elution can also be performed by boiling in denaturing SDS gel loading buffer instead of using biotin, if the isolated proteins are not needed in functional form.

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
Strep-tag® washing buffer (Buffer W)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA	2-1003-100
Biotin elution buffer BXT (Buffer BXT); 1x concentrated; 250 ml	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 50 mM D-biotin	2-1041-250
Magnetic Separator for 24 reaction tubes		2-1602-000

General Remarks

- Strep-Tactin "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 >10 µg/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 lower than 10 µg/ml, we highly recommend using our Strep-Tactin® purification columns.

Important notes

- Do not use Buffer BE for elution of the target protein from MagStrep "type3" XT beads because this might lead to incomplete elution results! Instead use buffer BXT (see above).
- In general, Twin-Strep-tag® leads to better yields in batch purification due to its higher 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 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.
- 2. Pipette the required amount of beads into a vial, place it on the magnetic separator to separate beads and remove 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 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 D-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 BX 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/technical-support.html for downloading this manual and for further information regarding “Reagents compatible with Strep-tag/Strep-Tactin interaction” and Troubleshooting Guides.



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