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Protocol

MagStrep® Strep-Tactin®XT bead-based protein purification

Fast purification of Strep-tag®II and Twin-Strep-tag® fusion proteins in reaction tubes with high purity

1 DESCRIPTION

MagStrep® Strep-Tactin®XT beads are ferrimagnetic spheres covered with 6% agarose and coupled with Strep-Tactin®XT allowing efficient immobilization and purification of Strep-tag®II as well as Twin-Strep-tag® fusion proteins. The magnetic core is used to easily separate the beads from the surrounding liquid with the help of a magnet, making MagStrep® Strep-Tactin®XT beads perfectly suitable for batch purifications. Due to the agarose surface and the high specificity of Strep-Tactin®XT, the beads show a very low non-specific protein binding.

This protocol describes the application of MagStrep® Strep-Tactin®XT beads for batch purification in reaction tubes (3.1) and 96-well plates (3.2).

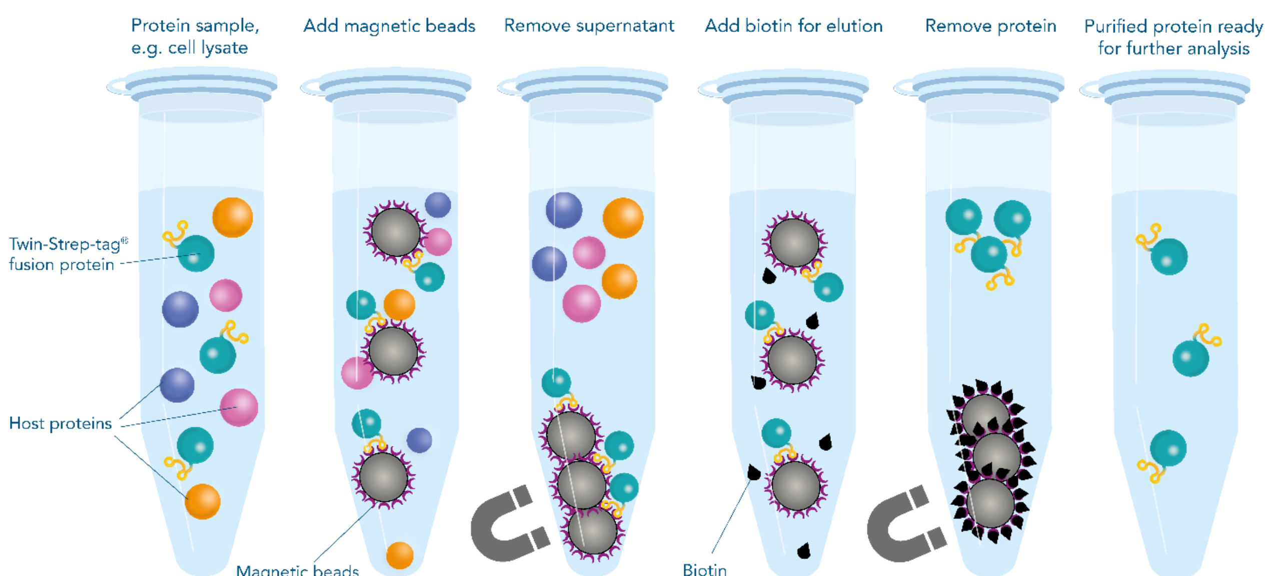

***Figure 1**: Workflow of protein purification with MagStrep® Strep-Tactin®XT beads*

2 REQUIRED PRODUCTS AND RECOMMENDATIONS

2.1 Required buffers

The table below lists all required buffers provided by IBA Lifesciences for protein purification with MagStrep® Strep-Tactin®XT beads (Cat. No. 2-5090-xxx). Please note that the buffers are provided as 10-fold concentrated solutions. Prior to application, the buffers have to be diluted to obtain single concentrated buffers. To achieve this, mix one part of 10-fold concentrated buffer with nine parts of deionized water.

Required buffers for protein purification with MagStrep® Strep-Tactin®XT beads		Cat. No.
10x Buffer W (1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, pH 8)	100 ml	2-1003-100
10x Buffer BXT (1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, 500 mM biotin, pH 8)	25 ml	2-1042-025

MagStrep® Strep-Tactin®XT beads can be regenerated with 0.1 M freshly prepared NaOH (regeneration buffer). The regeneration buffer is not provided by IBA Lifesciences.

2.2 Recommendations

Using the Twin-Strep-tag® leads to higher yields compared to Strep-tag®II fusion proteins, due to the higher binding affinity to Strep-Tactin®XT.

You can adjust the volume of magnetic beads according to your target protein concentration.

For proteins >90 kDa, optimal binding might not be reached under the described conditions.

More information on how to improve your protein yield can be found in section 4.1.

For fast and simple separation of magnetic beads from buffer or sample, we recommend the application of our Magnetic Separator (Cat. No. 2-1602-000) if the protein purification occurs in reaction tubes (1.5-2 ml). With the Magnetic Separator up to 24 samples can be processed in parallel. If a larger number of samples should be purified, we recommend the use of round-bottom 96-well plates and an appropriate magnetic device.

3 PROTOCOL

3.1 Batch purification in reaction tubes

A video tutorial of the purification is available at https://www.youtube.com/watch?v=FEbuMCfZhb4

- This protocol is optimized for working at room temperature.
- 20 µl of the 5 % suspension contain 1 µl magnetic beads.
- The binding capacity of the magnetic beads is 42.5 µg/µl (0.85 nmol of a 50 kDa protein)
- The volume of the cleared extract should not exceed 2.5 ml per µl MagStrep® Strep-Tactin®XT beads.

3.1.1 Resuspend the magnetic beads by pipetting up and down. For **250 µl sample**, pipette **100 µl of the 5 % suspension** (5 µl beads) into a reaction tube, place it on the magnetic separator to separate the beads and remove the supernatant.

3.1.2 Equilibrate beads in **500 µl 1x Buffer W**. Separate the beads in the magnetic separator and remove the supernatant. Repeat this step two times.

3.1.3 Resuspend the magnetic beads with the **250 µl sample** containing the target protein. Incubate for **10 minutes**. Keep the beads in suspension by inverting the tube occasionally, or by placing the tube on a roller.

3.1.4 Place the reaction tube in the magnetic separator and carefully remove the supernatant.

3.1.5 Remove tube from the magnet and add **500 µl 1x Buffer W**. Resuspend carefully and place the reaction tube in the magnetic separator to collect the beads. Remove the supernatant and repeat this step two times.

3.1.6 Elution can occur under native (a) or denaturing (b) conditions. Elution under native conditions offers specific elution conditions and thereby higher protein purity.

a) **Native elution:** Remove the reaction tube from the magnetic separator, resuspend with **125 µl 1x Buffer BXT** and incubate for **10 minutes**. Place the reaction tube in the magnetic separator and transfer the supernatant containing the target protein into a new reaction tube. Repeat this step once for higher recovery. The first elution step will yield the target protein at the highest concentration.

b) **Denaturing elution:** Apply conventional SDS-PAGE sample buffer and heat the sample to **95 °C** for **2 minutes**. Immobilized Strep-Tactin®XT will denature under these conditions and detach from the magnetic beads, 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.

3.2 Batch purification in 96-well plates

- Use a round bottom well plate.

3.2.2 Pipette at least **40 µl of the 5% magnetic bead suspension** (corresponding to 2 µl magnetic beads) into each well.

3.2.3 Equilibrate beads in **100 µl 1x Buffer W per 1 µl beads**. Separate the beads in the 96-well plate magnetic separator and remove the supernatant. Repeat this step two times.

3.2.4 Add **200 µl sample** and incubate for **10 minutes** at room temperature by constantly shaking on a microplate orbital shaker at 700 rpm.

3.2.5 Place the microplate on the 96-well plate magnetic separator and carefully remove the supernatant.

3.2.6 Remove plate from the magnet and add **100 µl 1x Buffer W** per µl beads and place the microplate in the 96-well plate magnetic separator. Remove supernatant and repeat this step two times.

3.2.7 Add **50 µl 1x Buffer BXT per 1 µl beads**. Incubate by constantly shaking at 400 rpm for 10 minutes at room temperature. Place the microplate in the 96-well plate magnetic separator and transfer the supernatant containing the target protein into a new microplate. Repeat this step once for higher recovery. The first elution step will yield the target protein at the highest concentration.

3.3 Magnetic Bead Regeneration (optional)

Please note that the regeneration in batch may be incomplete if done incorrectly. After an incomplete regeneration, proteins from the first purification may remain on the magnetic beads and can be co-purified as an impurity once another protein is purified using the same beads. Especially in applications like pull-down assays or co-immunoprecipitations, remaining proteins could lead to false-positive hits. Therefore, we do not recommend using the regenerated magnetic beads for the purification of a different protein than the one they were originally used for.

3.3.1 Freshly prepare the regeneration buffer (0.1 M NaOH) before use.

3.3.2 Add **100 µl of regeneration buffer per 1 µl of magnetic beads**. Incubate for **2 minutes** at room temperature.

Place the beads in a magnetic separator to separate them. Remove the supernatant.

3.3.3 Equilibrate beads in **100 µl 1x Buffer W per 1 µl beads**. Separate the beads in the magnetic separator and remove the supernatant. Repeat this step two times.

3.3.4 Store the beads in **1x Buffer W** at **2-8 °C**.

4 OPTIMIZATION AND TROUBLESHOOTING

4.1 Optimization

Starting from the standard protocol described in section 3, binding efficiency can be optimized for each individual protein.

- If the protein concentration in the sample is known, adjust the bead volume accordingly. For optimal yield, the total bead binding capacity should be around 5 times the total amount of protein. For example, if the total amount of a 50 kDa protein is 85 µg, which theoretically could be bound by 2 µl of beads, add 5x 2 µl = 10 µl beads and incubate for 10 minutes to achieve the best yield.
- Samples with a low protein concentration should be concentrated before use, or a smaller bead volume should be added. The minimum recommended protein concentration is 1 pmol/µl (50 ng/µl of a 50 kDa protein).
- For large proteins >90 kDa, the binding capacity may decline. To improve the yield of large proteins, increase the bead volume.

For more detailed information please visit https://www.iba-lifesciences.com/applications/protein-purification/magnetic-beads-purification/

4.2 Troubleshooting

4.2.1 No or weak binding to MagStrep® Strep-Tactin®XT beads

Strep-tag®II or Twin-Strep-tag® is not present.	Add protease inhibitors during cell lysis and work quickly at 2-8 °C. If *E. coli* is used as expression host, use a protease deficient expression strain.
Strep-tag®II or Twin-Strep-tag® is not accessible.	Fuse the tag with the other protein terminus, use a different linker, or exchange the Strep-tag®II by Twin-Strep-tag®.
Strep-tag®II or Twin-Strep-tag® has been degraded.	Check if the tag is associated with a portion of the protein that is processed. If it is the case, change the position of the tag.
Strep-tag®II or Twin-Strep-tag® is partially accessible.	Reduce washing volume.

4.2.2 Contaminating proteins

Contaminants are short forms of the tagged protein.	Use protease deficient *E. coli* expression strains. Add protease inhibitors after cell lysis. Fuse Strep-tag®II with the other protein terminus. Check if internal translation initiation starts (only in case of C- terminal tag) or premature termination sites (only in case of N-terminal tag) are present. Add another tag to the other terminus and use both tags for purification.
Contaminants are covalently linked to the recombinant protein via disulfide bonds.	Add reducing reagents to all buffers. A list with compatible reagents can be downloaded at https://www.iba-lifesciences.com/downloadcenter/.
Contaminants are non-covalently linked to the recombinant protein.	Increase the ionic strength of all buffers (up to 5 M NaCl) or add mild detergents (up to 2% Triton X-100, 2% Tween 20, 0.1% CHAPS, etc.). A list with compatible reagents can be downloaded at https://www.iba-lifesciences.com/downloadcenter/.
Contaminants are biotinylated proteins.	Add BioLock (biotin blocking solution) or avidin. A detailed protocol for biotin blocking can be downloaded at https://www.iba-lifesciences.com/downloadcenter/.

Check our Downloads page

https://www.iba-lifesciences.com/downloadcenter/

for the latest version of this manual.

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If you have any questions, please contact

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We are here to help!

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