Cube Biotech

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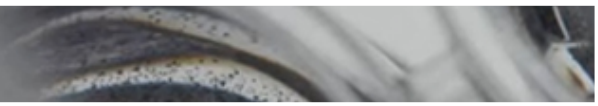
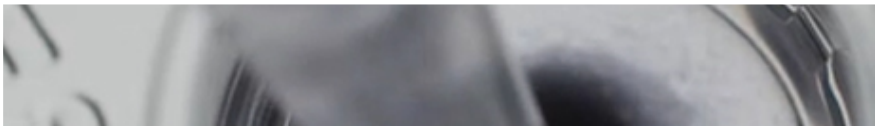
STEP-BY-STEP GUIDE: MAGNETIC BEAD-BASED PROTEIN PURIFICATION

Fast purification of Strep-tag®II and Twin-Strep-tag® fusion proteins with MagStrep® Strep-Tactin®XT beads



Save 30 minutes processing time on average!*



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INTRODUCTION

MagStrep® Strep-Tactin®XT beads are the ideal tool for quick and simple purification of Strep-tag® fusion proteins. They can be used for any type of protein, in small-scale experiments or in high-throughput purifications. Their magnetic core enables separation from the supernatant without centrifugation or the use of a column, making the batch purification workflow very straightforward. Additionally, MagStrep® beads can be used to study protein-protein interactions via pull-down assays. The fast and easy purification conserves even weak protein-protein interactions. For purification of proteins with tags other than Strep-Tag®, Cube Biotech offers different magnetic beads in their portfolio.

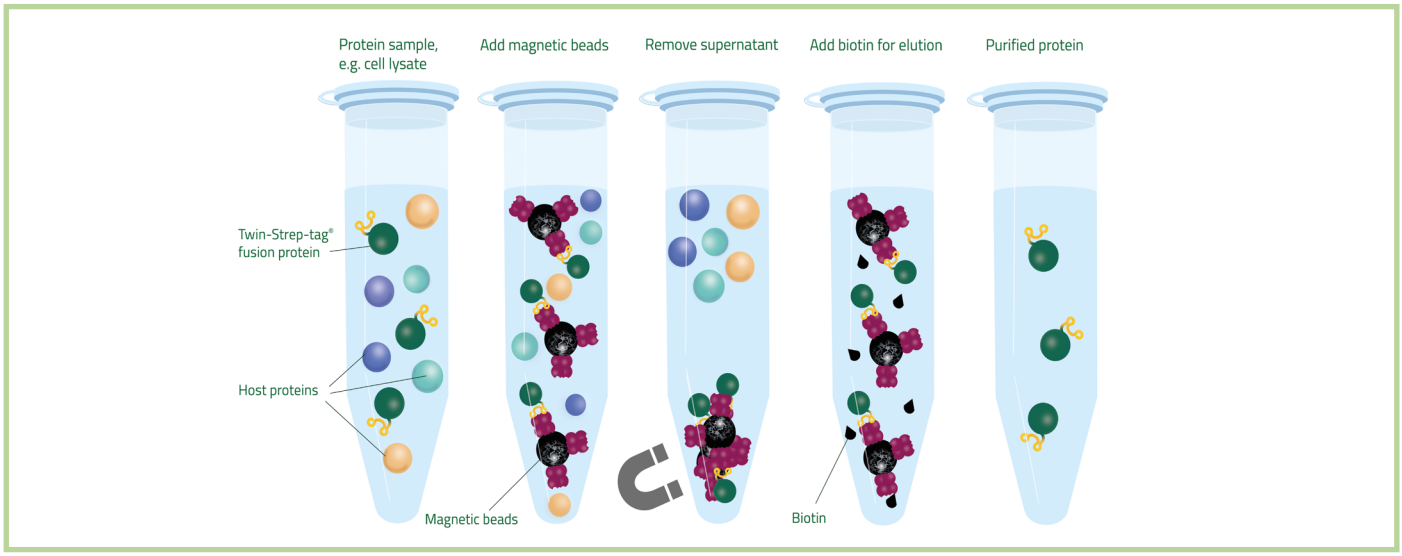

Figure 1: Workflow of protein purification with MagStrep® Strep-Tactin®XT beads

Specifications of the MagStrep® Strep-Tactin®XT beads	
Form	5% suspension
Binding capacity	42.5 mg/ml (measured with a 50 kDa protein)
Matrix	6% agarose, crosslinked, spherical magnetic beads
Bead diameter	30 µm average
Specificity	Strep-tag®II and Twin-Strep-tag®

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MATERIALS

2.1 Required materials

The table below lists all required buffers and the appropriate magnetic beads.

Required products for batch purification in reaction tubes		Cat. No.
MagStrep® Strep-Tactin®XT beads	2 ml 10 ml	2-5090-002 2-5090-010
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

Dilute buffers prior to application:
one part buffer with 9 parts deionized water.

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 on page 6.

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.

Check out our video tutorial:
https://www.youtube.com/watch?v=FE-buMCfZhb4

03

PROTOCOL

3.1 Batch purification in reaction tubes

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.

This protocol is optimized for working at room temperature.

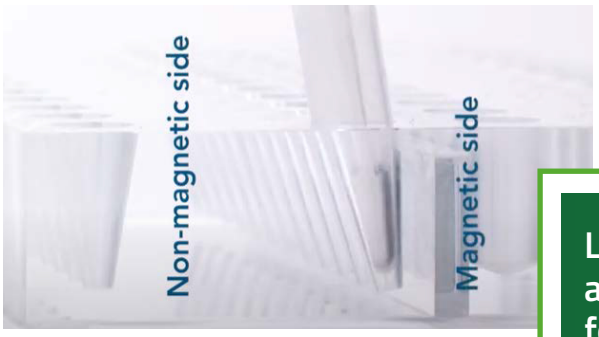
20 µl of the 5 % suspension contain 1 µl magnetic beads.

The volume of the cleared extract should not exceed 2.5 ml per µl MagStrep® Strep-Tactin®XT beads.

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.

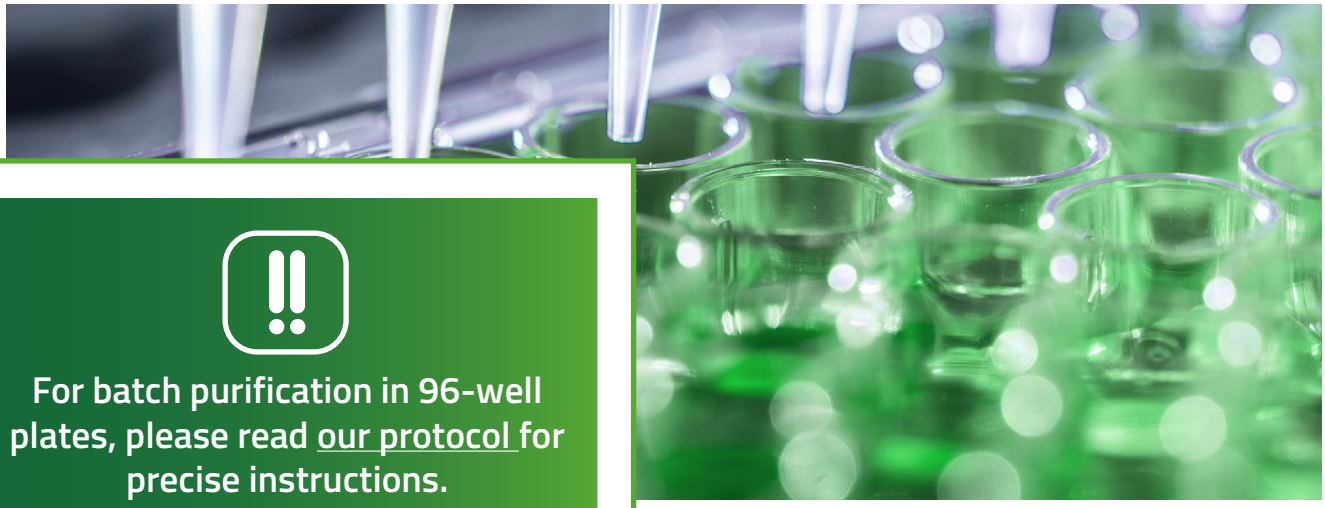


Let the magnet attract the beads for about 20 seconds.

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 or denaturing conditions. Elution under native conditions offers specific elution conditions and thereby higher protein purity.

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.
For denaturing conditions please check our protocol.



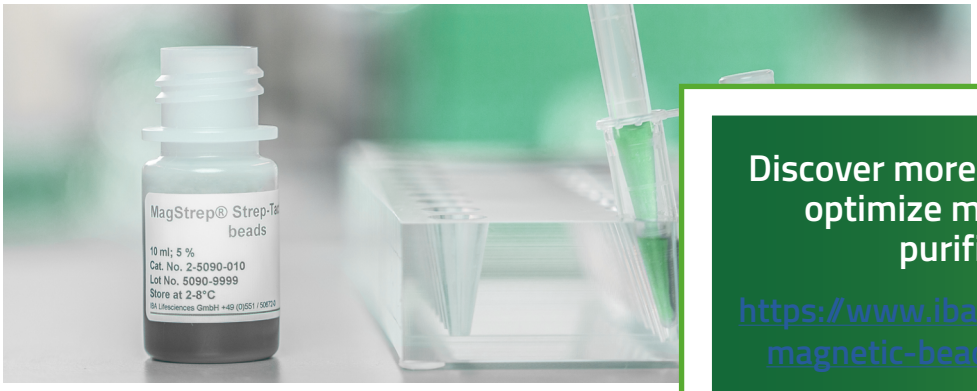
For batch purification in 96-well plates, please read our protocol for precise instructions.

04 OPTIMIZATION AND TROUBLESHOOTING

4.1 Optimization

Depending on your experimental conditions, it's possible to optimize the purification protocol and increase protein yield by adjusting specific parameters. The following tips below will help you optimize magnetic bead protein purification by adjusting bead volume, incubation time, and protein concentration.

1. **Required bead volume depends on the protein size:** In comparison to small proteins, large proteins need more space to bind whereby binding sites on the beads are not accessible for other proteins. Thus, purifying large proteins requires more beads to purify an equal number of proteins as for small proteins.
2. **Increased bead volumes reduce the incubation time:** An increase of the incubation time for several minutes can increase the amount of bound protein and consequently the protein yield.
3. **Sample protein concentration and bead volume must be balanced:** The more beads are used, the more target protein can be bound in a short time. In high concentrated samples, protein and bead can quickly find each other. This facilitates the purification of as much protein as possible.



Discover more data to help you optimize magnetic bead purification:

https://www.iba-lifesciences.com/magnetic-beads-optimization/

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.
Contaminants are noncovalently 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.).
Contaminants are biotinylated proteins	Add BioLock (biotin blocking solution) or avidin.

* on average in comparison to other magnetic beads available on the market.