Protein Production & Assays

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Protocol

MagStrep “type 3” XT bead-based protein purification

For fast purification of Strep-tag®II and Twin-Strep-tag® fusion proteins in reaction tubes or 96-well plates

1 DESCRIPTION

MagStrep “type3” 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 “type3” 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. MagStrep “type3” XT beads are supplied as 5% (v/v) suspension, which means that 20 µl of the homogenous suspension contains 1 µl magnetic beads. Per µl beads up to 0.85 nmol Strep-tag®II or Twin-Strep-tag® fusion protein can be purified, which corresponds to 25.5 µg of a 30 kDa protein in which the Twin-Strep-tag® leads to higher yields due to the higher binding affinity of Strep-Tactin®XT. This protocol describes the application of MagStrep “type3” XT beads for batch purification in reaction tubes (3.1) and in high-throughput screens with 96-well plates (3.2). Please note that the regeneration of the beads after the batch purification is incomplete and therefore the beads should not be reused.

2 REQUIRED PRODUCTS

In the table below all required buffers and the appropriate magnetic beads are listed. Please note that the buffers are provided as tenfold concentrated solution. Prior to application, the buffers have to be diluted to obtain single concentrated buffer. To achieve this, mix one part tenfold concentrated buffer with nine parts water.

Required products for batch purification in reaction tubes or 96-well plates		Cat. No.
MagStrep “type3” XT beads	2 ml 10 ml	2-4090-002 2-4090-010
10x Buffer W (1 M Tris-Cl, 1.5 M NaCl, 10 mM EDTA, pH 8)	100 ml	2-1003-100
10x Buffer BXT (1 M Tris-Cl, 1.5 M NaCl, 10 mM EDTA, 500 mM biotin, pH 8)	25 ml	2-1042-025

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-well bottom 96-well plates and an appropriate magnetic separator.

3 PROTOCOL

3.1 Batch purification in reaction tubes

- 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. 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.

3.1.1 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 which correspond to 25.5 µg of a 30 kDa protein.

3.1.2 Pipette the required amount of beads into a reaction tube, place it on the magnetic separator to separate the beads and remove the supernatant.

3.1.3 Equilibrate beads in 200 µl 1x Buffer W per µl beads. Separate the beads in the magnetic separator and remove the supernatant. Repeat this step two times.

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

3.1.5 Resuspend the magnetic beads with the appropriate volume of the cleared sample containing the target protein. Incubate 30 minutes on ice. Vortex three to four times during incubation to resuspend the beads.

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

3.1.7 Remove the magnet and add 100 µl 1x Buffer W per µl beads. Vortex shortly and place the reaction tube in the Magnetic Separator to collect the beads. Remove the supernatant and repeat this step two times.

3.1.8 Elution can occur under native (a) or denaturing (b) conditions. Please use one of these options. We recommend elution under native conditions due to the specific elution conditions and thereby higher protein purity.

a) Native elution: Remove the reaction tube from the Magnetic Separator and add 25 µl 1x Buffer BXT per µl beads and vortex. Incubate 10 min and vortex two to three times during this to resuspend the beads. 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. Analyze purity via SDS-PAGE and Coomassie or silver staining and quantify according to Bradford using BSA as standard.

b) Denaturing elution: Instead of 1x Buffer BXT apply conventional SDS-PAGE sample buffer and heat the sample to 95 °C for 2 min. Immobilized 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.

3.2 Batch purification in 96-well plates

- The size of the target protein should not exceed 90 kDa to get a good binding efficiency.

3.2.1 Determine the target protein concentration in the sample. The concentration should be at least 0.425 nmol/200 µl sample.

3.2.2 Pipette at least 40 µl of the 5% magnetic bead suspension (corresponding to 2 µl magnetic beads) into each well. Use 1 µl magnetic beads per 0.85 nmol recombinant protein which correspond to 25.5 µg of a 30 kDa protein.

3.2.3 Equilibrate beads in 100 µl 1x Buffer W per 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 60 min 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 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 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.

4 TROUBLESHOOTING

4.1 No or week binding to MagStrep “type 3” 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 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/download-area-protein.html.
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/download-area-protein.html.
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/download-area-protein.html.

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If you have any questions, please contact

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We are here to help!

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