Protein Production & Assays

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Protocol

Purification of Biotinylated Proteins with Strep-Tactin®

For Proximity-dependent Biotin Identification (BioID)

1 GENERAL INFORMATION

Originally Strep-Tactin®, an engineered streptavidin, was developed for efficient but reversible binding of a peptide that can be used for protein purification – the Strep-tag®. The binding of the Strep-tag® occurs via the engineered biotin-binding pockets of Strep-Tactin®. This Strep-tag®/Strep-Tactin® system is now widely used in the field of protein purification.
Nevertheless, Strep-Tactin® still has the capability to bind biotin, which is used as specific competitor for elution of Strep-tagged proteins. The binding affinity of biotin to Strep-Tactin® is not as high as for streptavidin and, therefore, biotinylated molecules can bind to Strep-Tactin® in a reversible manner. The release from binding is carried out under mild and physiological conditions by adding an excess of free biotin. This reversible binding to Strep-Tactin® is applicable for purification, detection, and immobilization of biotinylated molecules. Thus, it extends the field of application to the characterization of protein complexes and mapping of protein-protein interactions using proximity-dependent biotin identification (BioID)[3].
BioID involves the expression of a promiscuous biotin ligase BirA* fused to the protein of interest (bait), leading to biotinylation of lysine residues of nearby interactors (prey). Due to the affinity of Strep-Tactin® for biotin, biotinylated interactors can be easily enriched with Strep-Tactin® resins and, afterwards, analyzed by Western blot or mass spectrometry[1, 2].

2 MATERIALS FOR PURIFICATION OF BIOTINYLATED PROTEINS

IBA Lifesciences offers several resins coupled with Strep-Tactin® compatible for purification of biotinylated proteins. An overview is given in the following table. Please note that Strep-Tactin®XT resins are not recommended for purification of biotinylated proteins. In comparison to Strep-Tactin®, Strep-Tactin®XT has a lower affinity for biotin leading to an inefficient immobilization and purification of biotinylated proteins.

Compatible resins for purification of biotinylated proteins		Cat. No.
Strep-Tactin® Sepharose® resin	4 ml 20 ml 50 ml	2-1201-002 2-1201-010 2-1201-025
Strep-Tactin® Sepharose® column	5 x 0.2 ml resin bed volume 1 ml resin bed volume 5 ml resin bed volume 10 ml resin bed volume	2-1202-550 2-1202-001 2-1202-051 2-1202-101
Strep-Tactin® Superflow® resin	50 ml	2-1206-025
MagStrep “type 3“ beads with Strep-Tactin®	2 ml	2-1613-002

For washing and elution, the application of 1x Buffer W with 0.4% SDS and 1x Buffer BXT is recommended. Both buffers are available as 10x concentrated stock solutions without SDS.

Recommended buffers for purification of biotinylated proteins		Cat. No.
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

Further buffers necessary for purification but not provided by IBA Lifesciences are listed in the table below.

Buffers not provided by IBA Lifesciences	Composition
SDS stock solution (20%)	For preparation of 1x Buffer W with 0.4% SDS. Dilute 10x Buffer W and add SDS before filling up with H_2O
Cell Washing Buffer	10 mM HEPES pH 7.9 10 mM KCl 1.5 mM $MgCl_2$
Cell Lysis Buffer	10 mM HEPES pH 7.9 10 mM KCl 1.5 mM $MgCl_2$ add freshly 0.5 mM DTT and protease inhibitor (PI)
2x SDS-PAGE sample buffer:	0.17 M Tris-HCl pH 6.8 20% glycerol 4.7% SDS 0.2% bromphenol blue 10% β-mercaptoethanol

3 SAMPLE PREPARATION

3.1 Cultivation and harvest of *Saccharomyces cerevisiae*

3.1.1 Grow a *Saccharomyces cerevisiae* main culture (150 ml) with 10 µM biotin overnight until the optical density at 600 nm (OD_{600}) reached 0.8. The appropriate culture volume might vary depending on the expression rate of the bait protein fused to the biotin ligase.

3.1.2 Harvest the cells at 4 °C and remove supernatant.

3.1.3 Freeze in liquid nitrogen and store at -80 °C, or directly proceed with the washing of cells.

3.2 Washing of cells

3.2.1 Thaw cells on ice and work on ice until SDS is added to the samples.

3.2.2 Resuspend cell pellet in 10 ml Cell Washing Buffer.

3.2.3 Spin down the cells at 4 °C and remove the supernatant.

3.2.4 Repeat washing with 10 ml Cell Washing Buffer.

3.2.5 Resuspend cells in 1 ml Cell Washing Buffer and transfer the suspension to a screw cap tube.

3.2.6 Spin down at 1.500 x g for 45 sec and 4 °C to pellet the cells. Remove the supernatant.

3.3 Cell lysis

3.3.1 Prepare 450 µl Cell Lysis Buffer for each sample. Cell Lysis Buffer and protocol might need adjustment according to the used cell system.

3.3.2 Resuspend cells in 250 µl Cell Lysis Buffer, add cold glass beads, and disrupt cells through vigorous shaking.

3.3.3 Add 200 µl Cell Lysis Buffer and 112.5 µl 20% SDS. The final proportion of SDS in the sample should be 4%.

3.3.4 Continuously shake the sample for 10 min and then heat at 65 °C for 5 min for complete cell lysis.

3.3.5 Centrifuge for 5 min, at 16.200 x g and room temperature. Transfer the supernatant to a new microcentrifuge tube. Repeat the centrifugation step until the lysate appears clear.

3.3.6 Take 40 µl as input control and a further aliquot for determination of protein concentration. Store both samples at -20 °C until analysis.

3.3.7 Proceed with batch purification protein purification via (**4.1**) or gravity flow columns (**5.1**).

4 BATCH PURIFICATION OF BIOTINYLATED PROTEINS WITH STREP-TACTIN® BEADS

4.1 Equilibration of the beads

4.1.1 Prepare 750 µl 1x Buffer W per sample.

4.1.2 For a sample derived from 150 ml cell culture use either 100 µl Strep-Tactin® Sepharose® or 1 ml MagStrep “type 3“ beads with Strep-Tactin® to obtain a bed volume of 50 µl.

4.1.3 Centrifuge for 2 min at 400 x g and room temperature or in case of the use of magnetic beads place the reaction tubes into the magnetic separator. Carefully remove the supernatant to avoid loss of beads.

4.1.4 Wash beads with 375 µl 1x Buffer W. Centrifuge again with the same adjustments and, afterwards, remove the supernatant. Repeat the washing step.

4.2 Capture of biotinylated proteins and wash of the beads

4.2.1 Add the cleared cell lysate to the equilibrated beads and incubate for 30 min under continuous agitation. Ensure that the liquid is in contact with the beads.

4.2.2 Centrifuge (400 x g, 2 min, room temperature) or place the reaction tubes into the magnetic separator. Collect the supernatant as unbound fraction and store the sample at -20 °C until analysis.

4.2.3 Prepare 2.4 ml 1x Buffer W with 0.4% SDS per sample. Please note that fast washing will improve the protein yield.

4.2.4 Wash each sample three times with 800 µl 1x Buffer W with 0.4% SDS (add buffer and shake it briefly). Centrifuge (400 x g, 2 min, room temperature) or place the reaction tubes into the magnetic separator between each washing step and collect the wash fractions as additional controls. Store the sample at -20 °C until analysis.

4.3 Elution of the biotinylated proteins by biotin

Elution with biotin is recommended due to the specific elution conditions. Nevertheless, if elution by boiling is preferred, please proceed with section 4.4

4.3.1 Please note, for elution of biotinylated proteins 1x Buffer BXT containing 50 mM biotin is recommended. Alternatively, equivalent buffers with 10-50 mM biotin are applicable as well.

4.3.2 Prepare 150 µl 1x Buffer BXT per sample.

4.3.3 Add 75 µl 1x Buffer BXT and incubate 10 min under occasional agitation. Ensure that the liquid is in contact with the beads. Centrifuge (400 x g, 2 min, room temperature) or place the reaction tubes into the magnetic separator and transfer the elution fractions to a new microcentrifuge tube. Store the sample at -20 °C until analysis.

4.3.4 Repeat the elution step to increase the yield (total elution volume is 150 µl). Optional: The efficiency of the elution with 1x Buffer BXT can be evaluated by a second elution via boiling. Therefore, add 150 µl 2x SDS-PAGE sample buffer to the beads and heat to 95 °C for 3 min. Store the sample at -20 °C until the analysis.

4.3.5 Mix the samples after elution with biotin with SDS-PAGE sample buffer to load it onto an SDS-gel for subsequent Western blot experiments or in-gel digestion of proteins with trypsin for liquid chromatography-mass spectrometry (LC-MS) analysis. Alternatively, proteins can be digested in solution as well. Optional: Proteins can be precipitated, e.g., by chloroform-methanol extraction to increase protein concentration for SDS-PAGE.

4.4 Elution of the biotinylated proteins by boiling

Please note, under denaturing conditions Strep-Tactin® does not remain on the matrix and is present in the sample.

4.4.1 Prepare 150 µl 2x SDS-PAGE sample buffer per sample.

4.4.2 Add 150 µl 2x SDS-PAGE sample buffer to the beads and heat to 95 °C for 3 min.

4.4.3 Centrifuge (400 x g, 2 min, room temperature) or place the reaction tubes into the magnetic separator and transfer the elution fractions to a new microcentrifuge tube.

4.4.4 Store the sample at -20 °C until analysis.

5 PURIFICATION OF BIOTINYLATED PROTEINS WITH STREP-TACTIN® GRAVITY FLOW COLUMNS

5.1 Equilibration of the gravity flow column

5.1.1 Degas buffer W and keep at room temperature. Prepare at least 4 column bed volumes (CV) 1x Buffer W per column. That means for a 1 ml column 4 ml 1x Buffer W.

5.1.2 Remove top and lower cap a from the column at 4 °C and let the storage buffer pass through the column.

5.1.3 Add 2 CV degassed 1x Buffer W (room temperature) onto the column and adjust the column to room temperature (this should prevent formation of air bubbles due to the temperature shifts). From now on, all steps are performed at room temperature.

5.1.4 Equilibrate again with 2 CV degassed 1x Buffer W.

5.2 Capture and wash of the biotinylated proteins

5.2.1 Apply the cell lysate onto the column.

5.2.2 Collect the flow-through as control in a microcentrifuge tube. Store the sample at -20 °C until analysis.

5.2.3 Prepare at least 50 CV 1x Buffer W with 0.4% SDS per column.

5.2.4 Wash the gravity flow column with 50 CV 1x Buffer W containing 0.4% SDS. The use of the WET FRED sample applicator is recommended. Optional: Collect the wash fractions as control. Store the sample at -20 °C until analysis.

5.3 Elution of the biotinylated proteins

5.3.1 Please note, for elution of biotinylated proteins 1x Buffer BXT is recommend. Alternatively, equivalent buffers with 10-50 mM biotin are applicable as well.

5.3.2 Prepare at least 3 CV 1x Buffer BXT per column.

5.3.3 Elute with 3 CV 1x Buffer BXT.

5.3.4 Collect elution fractions in a microcentrifuge tube and store the samples at -20 °C until the analysis. Optional: Precipitate the proteins and dissolve the pellet in a smaller volume to increase protein concentration for SDS-PAGE.

6 REFERENCES

[1]Schmitt K, Valerius O. yRACK1/Asc1 proxiOMICs – Towards Illuminating Ships Passing in the Night. Cells. 2019 Nov 4;8(11):1384. doi: 10.3390/cells8111384. PMID: 31689955; PMCID: PMC6912217.

[2]Opitz N, Schmitt K, Hofer-Pretz V, Neumann B, Krebber H, Braus GH, Valerius O. Capturing the Asc1p/Receptor for Activated C Kinase 1 (RACK1) Microenvironment at the Head Region of the 40S Ribosome with Quantitative BioID in Yeast. Mol Cell Proteomics. 2017 Dec;16(12):2199-2218. doi: 10.1074/mcp.M116.066654. Epub 2017 Oct 5. PMID: 28982715; PMCID: PMC5724181.

[3]Roux KJ, Kim DI, Raida M, Burke B. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J Cell Biol. 2012 Mar 19;196(6):801-10. doi: 10.1083/jcb.201112098. Epub 2012 Mar 12. PMID: 22412018; PMCID: PMC3308701.

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If you have any questions, please contact

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