Protein Production & Assays

Application Note

SPECIFIC ELUTION AND AVOIDANCE OF CONTAMINATIONS IN BioID

Strep-Tactin® as alternative to Streptavidin

Introduction

The method of Biotin Identification (BioID) enables the identification of proteins in the neighborhood of a protein of interest (in the following called bait protein[3]). The bait protein is fused to a biotin ligase of bacterial origin (substrate-unspecific and promiscuous upon mutation) and the fusion protein causes biotinylation of proteins in the microenvironment of the bait. The biotinylated proteins are enriched from cell lysates through affinity purification prior to identification, e.g. with mass spectrometry. Matrices coated with streptavidin, a protein with natural high affinity for biotin, are commonly used for the enrichment **(Fig. 1)**. Proteins can be eluted through denaturation of the streptavidin, e.g. by boiling in SDS-PAGE sample buffer, which also releases streptavidin from the matrix. Streptavidin-derived peptides unfavorably compete with peptides of biotinylated proteins during Liquid Chromatography–Mass Spectrometry (LC-MS) analysis. To avoid this contamination, streptavidin variants with slightly reduced biotin affinity can be used for the enrichment of biotinylated proteins, namely Strep-Tactin® and Strep-Tactin®XT that have been originally developed to bind proteins with a short peptide tag, the Strep-tag®II, or the tandem version of it, the Twin-Strep-tag®. In contrast to streptavidin, efficient elution of biotin-bound proteins from Strep-Tactin® and Strep-Tactin®XT is possible with an excess of biotin[1,2]. While biotinylated proteins are eluted, Strep-Tactin® as well as Strep-Tactin®XT remain at the matrix and do not contaminate the sample.
This application note describes the comparison of matrices for BioID capture showing that Strep-Tactin® is best suited for efficient protein-biotin capture and elution in combination with biotin-containing buffers.

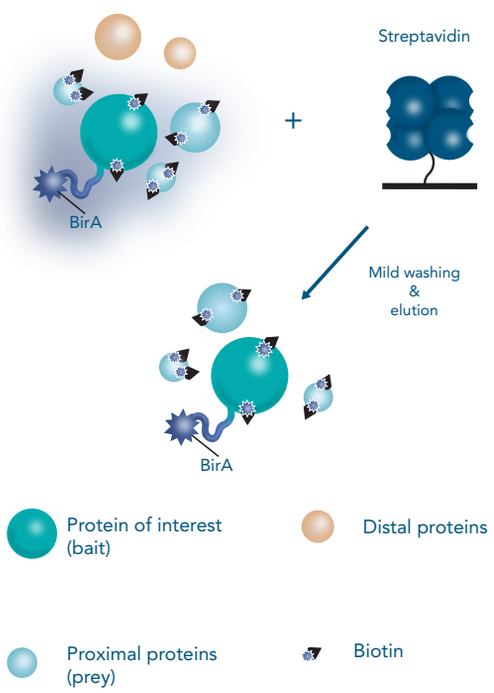
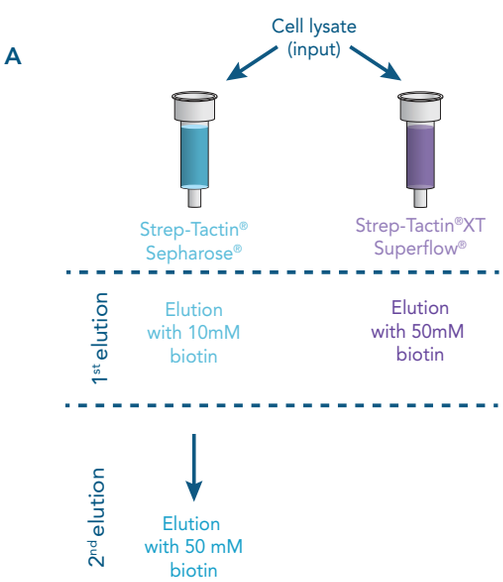


Fig. 1 Principle of BioID. The biotin ligase (BirA) is fused to the protein of interest (bait) and biotinylates the bait as well as transient and proximal interactors (prey). Afterwards, the biotinylated proteins are purified from cell lysates via affinity chromatography resins commonly coupled with streptavidin.

Results and discussion

Strep-Tactin® outperforms Strep-Tactin®XT regarding the enrichment of biotinylated proteins
First, we analyzed whether Strep-Tactin® or Strep-Tactin®XT is better suited to enrich biotinylated proteins from cell lysates. We used cell lysates with a relatively high abundance of biotinylated proteins in comparison to cell lysates of wild-type yeast strains. Therefore, we used a yeast strain that expresses an Asc1-BirA* fusion protein that causes a high degree

of protein biotinylation at ribosomes[2]. Yeast cells were cultivated in the presence of biotin to OD_{600} 0.8 in a total culture volume of 1.2 l. Cell lysates were prepared as described, and SDS was added to a final concentration of 4% for the denaturation of proteins. Cell lysates were split into equal halves, one applied to an equilibrated gravity flow column with Strep-Tactin® Sepharose® and the second one to a gravity flow column with Strep-Tactin®XT Superflow® (both columns with 0.2 ml bed volume, **Fig. 2A**). The flow through was collected and the column was washed five times with 2 ml of 1x Buffer W containing 0.4% SDS. The wash fractions 1 to 5 were collected. Biotin-bound proteins were eluted from the columns by adding 600 µl biotin elution buffer containing 10 mM biotin for Strep-Tactin® columns and 50 mM biotin for Strep-Tactin®XT columns, respectively. For the Strep-Tactin® column a second elution step with 50 mM biotin elution buffer was performed to analyze whether 10 mM biotin is sufficient for elution. Fractions of the purification process were mixed with 3x SDS-PAGE sample buffer and subjected to SDS-PAGE followed by western blot-like visualization of biotinylated proteins with Streptavidin-HRP. An aliquot of the input containing 50 µg of protein was loaded and an equal volume of the flow through and the wash fractions. From the eluate fractions 20 µl were used for SDS-PAGE. As shown in **Fig. 2B and C**, Strep-Tactin® outperformed Strep-Tactin®XT, and 10 mM biotin in the elution buffer seems to be sufficient to elute the majority of biotinylated proteins from the Strep-Tactin® column.



Strep-Tactin® Sepharose® is as powerful resin for enrichment of biotinylated proteins and boiling of beads for protein elution can be avoided

Often sample amount is limited, and it is thus crucial to get maximum yields upon protein enrichment. We compared the efficiency of capture with either Strep-Tactin® Sepharose® or Streptavidin Sepharose® (#17-5113-01, Streptavidin Sepharose® High Performance, Cytiva). Again, we used a cell lysate of an Asc1-BirA* expressing strain (derived from 450 ml yeast culture). For this test, we did batch processing with Sepharose® beads. The cell lysate was divided into three equal parts and incubated with

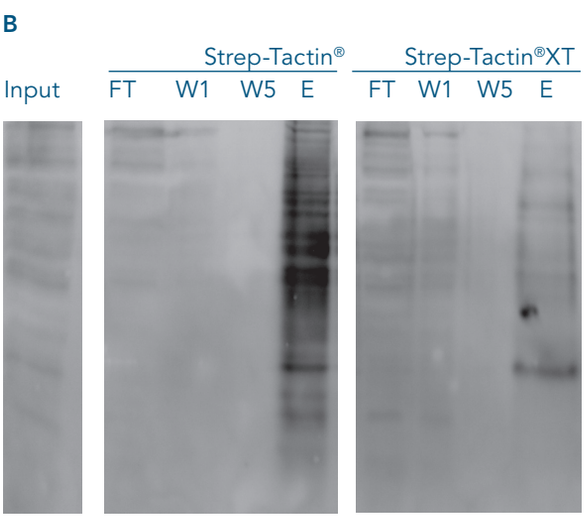


Fig. 2 Comparison of Strep-Tactin® and Strep-Tactin®XT for enrichment of biotinylated proteins. (A) Scheme of the workflow. **(B)** Protein fractions of the purification process were subjected to SDS-PAGE followed by western blot-like visualization of biotinylated proteins with Streptavidin-HRP. **(C)** Eluates of Strep-Tactin® columns after 10 mM and 50 mM biotin application (E1 and E2, respectively). FT = flow through, W1 = 1st wash fraction, W5 = 5th and last wash fraction, E = eluate.

Strep-Tactin® or Streptavidin Sepharose® (50 µl bed volume each) for 30 min during rotation. Afterwards, the samples were centrifuged and the supernatants containing unbound proteins were removed. The beads were washed 3 times with 1x Buffer W. Bound proteins were eluted through boiling in 150 µl SDS-PAGE sample buffer or by incubations with 50 mM biotin elution buffer (twice 75 µl for 10 min). **(Fig. 3A)**. Input aliquots (55 µg), flow-through (same volume as input), and elution samples (8 µl, however, for the biotin elution sample 12 µl were loaded because 8 µl of the sample had to be mixed with 4 µl 3x SDS-PAGE sample buffer prior to loading) were subjected to SDS-PAGE followed by membrane-blotting and biotin detection with Streptavidin-HRP. The results show that enrichment with Strep-Tactin® is highly efficient and that both elution strategies (boiling in SDS sample buffer and elution with biotin buffer) works equally well. The elution with biotin buffer has the advantage that Strep-Tactin® is absent from the elution fractions (see Ponceau stain, last lane C2 BXT, **Fig. 3B**). The enrichment with Strep-Tactin® performed even better than with Streptavidin. However, the protocol was originally optimized for the use of Strep-Tactin® [2] and might need improvement for the use of Streptavidin.

The eluate fractions were further subjected to SDS-PAGE for trypsin in-gel digestion of proteins[4] and LC-MS analysis. 30 µl of each of the two samples from the elution with SDS-PAGE sample buffer were loaded. For the biotin elution sample chloroform-methanol precipitation of proteins[5] was performed prior to SDS-PAGE. The dried protein sample was reconstituted in 8 M urea/ 2 M thiourea mixed with SDS-PAGE sample buffer and loaded on the gel. Samples were analyzed using a *Q Exactive* HF mass spectrometer (Thermo Fisher Scientific) and raw data searched against a *Saccharomyces cerevisiae*-specific protein database (UniProt) using the *Proteome Discoverer* 2.2 software (Thermo Fisher Scientific). In all three samples, the bait protein Asc1-BirA* was identified together with known co-localizing proteins such as Scp160 or Xrn1 (**Fig. 4**)[2]. The biotin eluted sample of Strep-Tactin® beads had the highest number of identified proteins **(Fig. 4)**. In summary, all three approaches can be used to successfully perform BioID experiments. The advantage of the elution with biotin is the reduction or even absence of Strep-Tactin® contamination. Due to the high affinity of streptavidin to biotin, competitive elution with a biotin containing buffer is not possible. Therefore, elution of biotinylated proteins only takes

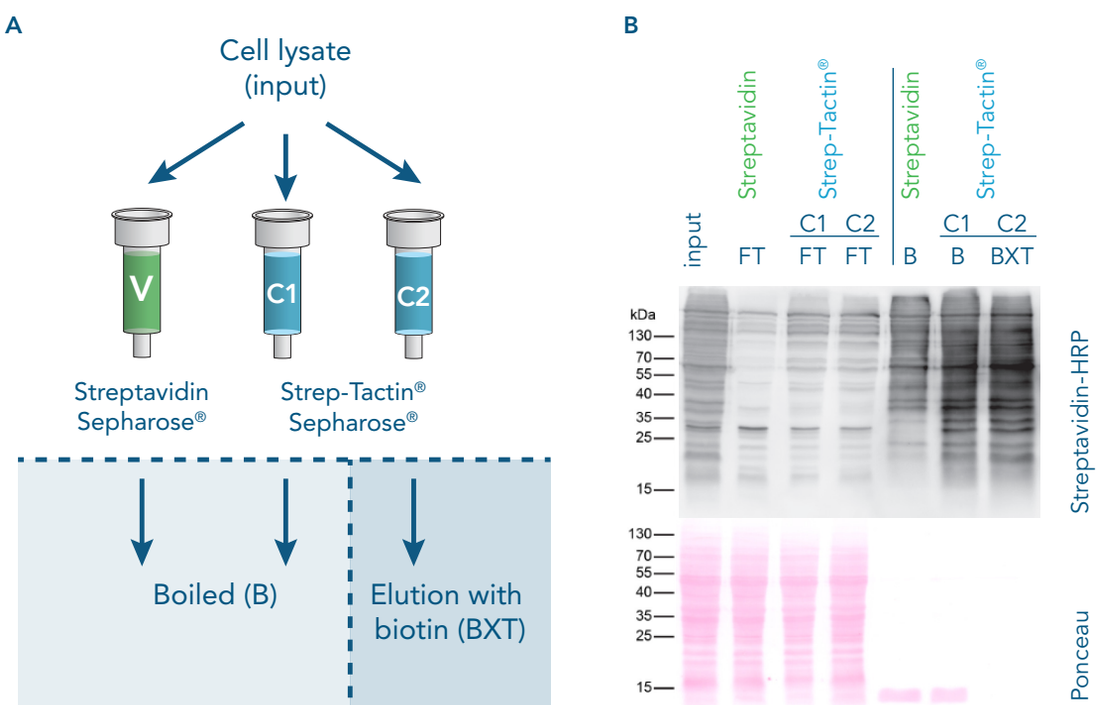


Fig. 3 Comparisons of Streptavidin Sepharose® and Strep-Tactin® Sepharose® for enrichment of biotinylated proteins. **(A)** Scheme of the workflow. **(B)** Protein samples collected during the capture process were subjected to SDS-PAGE followed by western blot-like detection of biotinylated proteins with Streptavidin-HRP. Protein transfer from gel to membrane was controlled by Ponceau staining of the membrane after blotting. V = Streptavidin Sepharose®, C1 and C2 Strep-Tactin® Sepharose® batch 1 and 2, FT = flow through, BXT = elution with 50 mM biotin (2 x 10 min incubation), B = elution by boiling (B) with SDS sample buffer for 3 min at 95°C

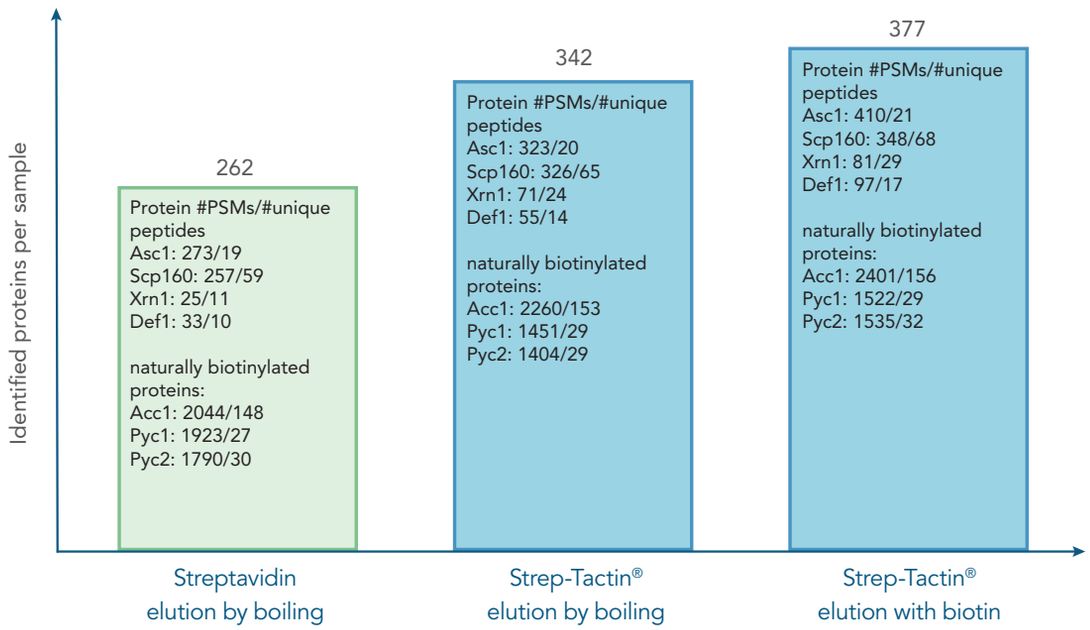


Fig. 4 Result of the LC-MS analysis to compare the use of Strep-Tactin® Sepharose® and Streptavidin Sepharose® for enrichment of biotinylated proteins. LC-MS data were analyzed using the *Proteome Discoverer* 2.2 software and data were filtered for proteins identified with at least two high-confident peptides (false discovery rate = 0.01). The bar chart shows the number of identified proteins per sample, and in each bar the number of peptide spectrum matches (PSMs) and unique peptides for Asc1, three of its known neighbors (Scp160, Xrn1, Def1; Opitz et al., 2017) and for three naturally biotinylated proteins (Acc1, Pyc1, Pyc2) are provided.

place via boiling leading to streptavidin contaminations. Moreover, larger protein fractions (e.g. deriving from enrichments using gravity flow columns with 1 ml bed volume) can be precipitated after elution for either SDS-PAGE gel loading and trypsin in-gel digestion or for digests in solution.

Different carrier materials perform equally well for biotin-affinity capture of proteins

We further tested different Strep-Tactin® coated materials for their efficiency in BioID experiments. Strep-Tactin® Sepharose®, Strep-Tactin® Superflow® and Strep-Tactin® magnetic beads were used as already described. Proteins were eluted using biotin, and beads were subsequently boiled with SDS-PAGE sample buffer to evaluate elution efficiency **(Fig. 5A)**. For Strep-Tactin® Sepharose® and Superflow® beads, one replicate was immediately subjected to protein elution with SDS-PAGE sample buffer to compare the efficiency of elution with biotin. Aliquots from the input, the unbound fractions and the eluates were subjected to SDS-PAGE and western blotting to detect biotinylated proteins with Streptavidin-HRP **(Fig. 5B)**. Elution efficiency of the biotin buffer was comparable with that after boiling. The second elution by boiling showed that nearly all proteins were eluted in the first step with biotin buffer. All three types of matrices coated with Strep-Tactin® successfully and efficiently enriched biotinylated proteins.

Conclusion

For the enrichment of biotinylated proteins in BioID experiments, Strep-Tactin® is a preferable alternative to Streptavidin. Strep-Tactin® offers the advantage to elute bound biotinylated proteins with biotin-containing buffers thus avoiding Strep-Tactin® contamination of the samples. Protein samples from Streptavidin-based enrichments contain Streptavidin contamination after matrix-treatment with SDS-PAGE sample buffer. Furthermore, proteins of eluate fractions from Strep-Tactin® enrichments can easily be precipitated or concentrated for in-solution digest with trypsin and subsequent LC-MS analysis.

Materials and methods

Saccharomyces cerevisiae cells expressing Asc1-BirA*[2] were cultivated in minimal medium containing biotin (10 µM) to an OD_{600} of 0.8. Cells were lysed under denaturing conditions (0.4% SDS) and protein concentrations were determined with BCA assays (#23224 and #23228, Pierce™ BCA Protein Assay Reagent A and B, Thermo Fisher Scientific). Cell extracts were

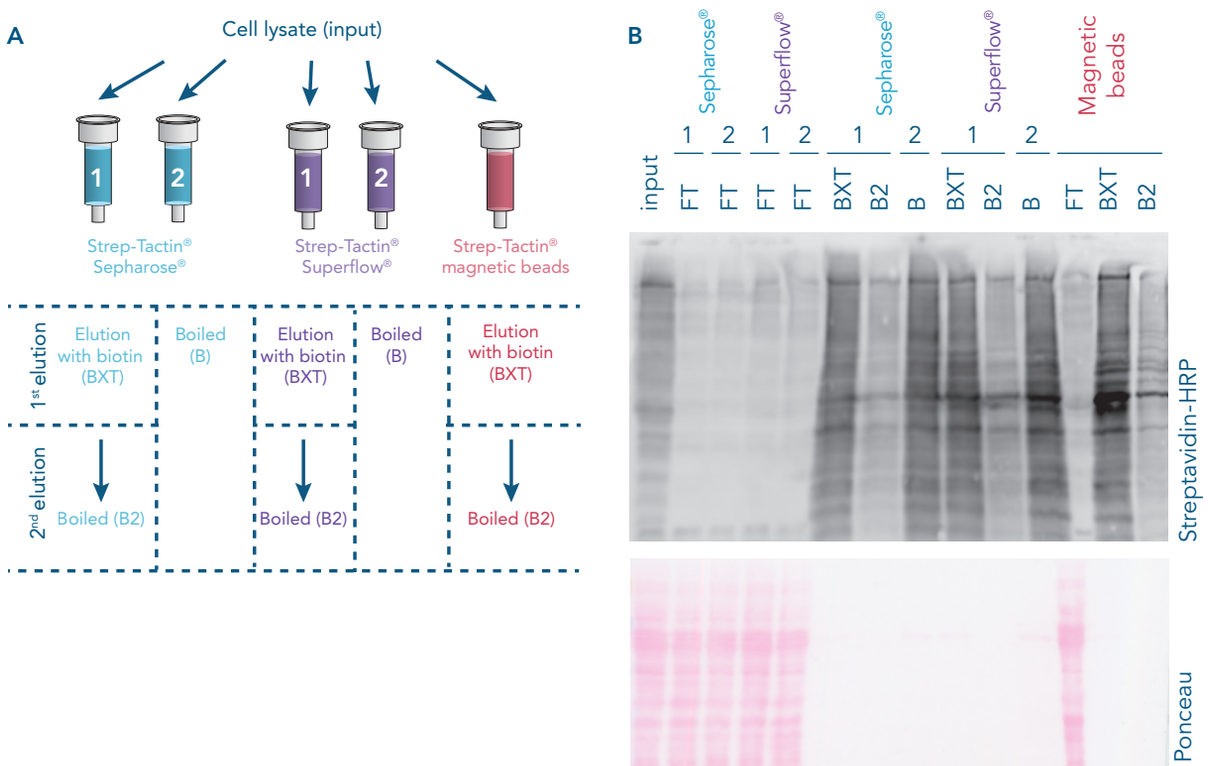


Fig. 5 Comparison of different carrier materials for biotin-affinity capture of proteins. (A) Workflow scheme. **(B)** Protein samples of the affinity purification were subjected to SDS-PAGE followed by western blot-like detection of biotinylated proteins with Streptavidin-HRP. Protein transfer from gel to membrane was controlled by Ponceau staining of the membrane after blotting. FT = flow through, BXT = elution with 50 mM biotin buffer, B = boiling with SDS sample buffer for 3 min at 95°C, B2 = control elution by boiling after elution with biotin buffer.

loaded onto either equilibrated gravity flow columns or equilibrated beads coated with Strep-Tactin®, Strep-Tactin®XT or streptavidin. After the cell lysate completely passed the column or after 30 min incubation with beads depending on the format of the matrix, matrices were washed using 1x Buffer W (100 mM Tris/HCl pH 8.0, 150 mM NaCl, 1 mM EDTA) containing 0.4% SDS. Proteins were eluted with biotin-containing buffers (100 mM Tris/HCl pH 8.0, 150 mM NaCl, 1 mM EDTA with 10 mM or 50 mM biotin) or through incubation of beads in SDS-PAGE sample buffer at 95 °C for 3 min. Fractions of the purification process were subjected to SDS-PAGE followed by western blot-like visualization of biotinylated proteins with Streptavidin-HRP (#21130, Pierce™ High Sensitivity Streptavidin-HRP, Thermo Fisher Scientific).

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