(Twin-) *Strep*-tag® Purification Short Protocol

Purification of (Twin-) *Strep*-tag® fusion proteins with *Strep*-Tactin® matrices

(Twin-) *Strep*-tag®/*Strep*-Tactin® affinity purification

The ***Strep*-tag®** purification system is based on the highly selective binding of engineered streptavidin, called *Strep*-Tactin®, to *Strep*-tag® II fusion proteins. This technology allows one-step purification of almost any recombinant protein under physiological conditions, thus preserving its biological activity. The *Strep*-tag® system can be used to purify functional *Strep*-tag® II proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria. Unique *Strep*-Tactin® affinity columns have been developed for this purpose and the corresponding operating protocol is described below. *Strep*-tag®/*Strep*-Tactin® affinity purification should not be performed discontinuously in batch mode which would result in significantly reduced protein purity and yield in comparison to column chromatography. Further, prolonged batch incubations increase the risk of proteolytic degradation of the target protein including cleavage of the tag. Because of its small size, *Strep*-tag® generally does not interfere with the biological activity of the fusion partner. Thus, removal of the tag becomes superfluous. Comprehensive reviews and scientific publications giving an overview of various *Strep*-tag® applications are listed at www.strep-tag.com.

The **Twin-Strep-tag®** is a dimeric version of the Strep-tag®II and therefore binds with the same selectivity to Strep-Tactin® but with a higher affinity. This higher affinity allows the purification of Twin-Strep-tagged proteins even from batch or cell culture supernatants with good yields. In addition the Twin-Strep-tag® tolerates higher amounts of detergents and salts in buffers compared to Strep-tag®II. Since the overall conditions for Strep-tag®II and Twin-Strep-tag® are the same, the following protocol can be used for both tags.

Short Protocol of the *Strep*-Tactin® chromatography cycle

Perform all operations at a temperature amenable to the stability of your recombinant protein (between 4 °C and 30 °C). To achieve optimal purification results, comply with the specified volumes and their ratios (column bed, washing volumes etc., see page 3). At low expression levels, increase applied cell extract volumes to take advantage of the column capacity, without changing other volumes.

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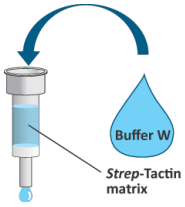
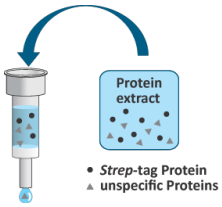
(Twin-) *Strep*-tag® Purification Short Protocol



Equilibrate the column

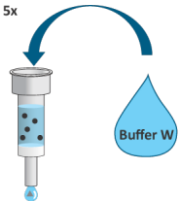
1. **Add 2 CV (column bed volume) Buffer W**



Apply the protein extract

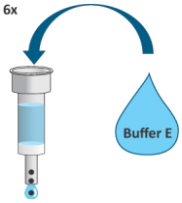
2. **Add 0.5 up to 10 CV cell extract**
   (Frozen cell extracts have to be centrifuged after thawing (14.000 rpm, 5 min, 4 °C) in order to remove any aggregates that may have formed. Apply the cleared extract to the column)

Wait until the cell extract has completely entered the column



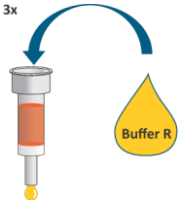
Wash column

3. **Wash column 5x with 1 CV of Buffer W**
   Collect the wash fractions (1 CV each) and apply 2 µl of each subsequent wash fraction to an analytical SDS-gel (additionally apply 2 µl of the lysate and 2 µl of the flow through).



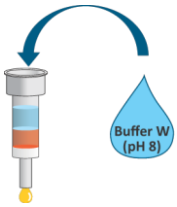
Elute protein

4. **Add 6x of 0.5 CV Buffer E**
   Collect the eluate in 0.5 CV fractions. To get most of *Strep*-tag® fusion protein in one fraction, i.e. E2, only, add 0.8 CV (E1), then 1.4 CV (E2) and finally again 0.8 CV (E3). 20 µl samples of each fraction can be used for SDS-PAGE analysis.



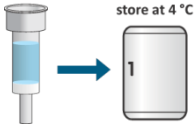
Regenerate column

5. **Wash the column 3x with 5 CV Buffer R**
   The color change from yellow to red indicates the regeneration process. The intensity of the red color is an indicator for the column activity status for the next round of purification.



Remove HABA and equilibrate

6. **Remove Buffer R by adding 2x of 4 CV Buffer W (pH 8)**
   This step is essentially the same for all Resins (*Strep*-Tactin Sepharose®, Macroprep® or Superflow® (high capacity)). If the column is not completely discolored after the removal step please wash with Buffer W at pH 10.5. Afterwards the columns have to be kept in **Buffer W at pH 8**.



Store column

7. **Store the column at 4 °C overlaid with 2 ml Buffer W (pH 8).**

Recommended volumes for working with *Strep*-Tactin® columns

Column bed volume (CV)	Protein extract volume*		Washing buffer volume	Elution buffer volume
	Strep-tagII	Twin-Strep-tag		
0.2 ml	0.1 – 2 ml	0.1 – 20ml	5 x 0.2 ml	6 x 0.1 ml
1 ml	0.5 – 10 ml	0.5 – 100 ml	5 x 1 ml	6 x 0.5 ml
5 ml	2.5 – 50 ml	2.5 – 500 ml	5 x 5 ml	6 x 2.5 ml
10 ml	5 – 100 ml	5 – 1000 ml	5 x 10 ml	6 x 5 ml

Table 1. Recommended buffer volumes for chromatography on *Strep*-Tactin® columns
*Adjust protein extract volume according to binding capacity of the column (please refer to the appropriate data sheet) and apply the extract as concentrated as possible in the recommended volume range. Note that these volumes are average values which can be different for certain proteins.

Biotin in cell culture media

Please note that biotin binds with high affinity to *Strep*-Tactin® thereby efficiently competing binding of (Twin-) *Strep*-tag®II. This binding is in addition irreversible and does to not allow regeneration of the *Strep*-Tactin® column (in contrast to bound desthiobiotin).
Especially media for mammalian cell or insect cell cultivation may contain significant amounts of biotin. Thus, if proteins are secreted to the culture medium, biotin must be masked by the addition of avidin (or biotin should be removed by dialysis or gel filtration) prior to *Strep*-Tactin® chromatography. For more information, particularly for a list enumerating the biotin content of different cell culture media, please refer to **www.iba-lifesciences.com/technical-support.html**.

Reagents compatible with the (Twin-) *Strep*-tag®/*Strep*-Tactin® interaction

For an overview of reagents, which have been successfully tested for the purification of e.g. GAPDH-*Strep*-tag® with concentrations up to those mentioned, see **www.iba-lifesciences.com/technical-support.html**.

For a more detailed protocol and troubleshooting please download the comprehensive *Strep*-tag manual under www.iba-lifesciences.com/technical-support.html. Here can also further *Strep*-tag related protocols be found for download.

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