

***Strep-tag*[®] Purification Protocol**

Purification of *Strep-tag* fusion proteins with *Strep-Tactin*[®] matrices

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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 protocols are described below.

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.iba-go.com. The comprehensive manual "Expression and purification of proteins using *Strep*-tag and/or 6xHistidine-tag" describing detailed protocols for various *Strep*-tag applications can be downloaded at "<http://www.iba-go.com/download.html>".

Protocol for affinity purification via *Strep*-Tactin affinity columns

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.). At low expression levels, increase applied cell extract volumes to take advantage of the column capacity, without changing other volumes.

Equilibration of *Strep*-Tactin columns

First remove the top then the bottom cap from the column and allow the excess storage buffer to drain off. Equilibrate the column by adding 2 CV (CV = column volume) of Buffer W (100 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA; use buffer without EDTA for metalloproteins). The column cannot run dry under gravity flow.

Adsorption of the *Strep*-tag II fusion protein and column washing

Add the cell extract having a volume between 0.5 and 10 CV to the column (concentrated cell extracts are preferable; if quantification is possible, apply cell extract containing between 50 and 100 nmol recombinant *Strep*-tag II fusion protein per 1 ml column bed volume). Frozen cell extracts have to be centrifuged before applying them to the column in order to remove any aggregates that may have formed (microcentrifuge, 14,000 rpm, 5 min., 4 °C). After the cell extract has completely entered the column, wash the column 5 times with 1 CV of Buffer W. Collect the wash fractions (1 CV each) and apply 2 µl of the first and 20 µl of each subsequent wash fraction to an analytical SDS gel (apply also 2 µl of the lysate and 2 µl of the flow through).

Elution of the recombinant protein

Add 6 times 0.5 CV Buffer E (= Buffer W containing 2.5 mM desthiobiotin as a reversibly binding specific competitor) and collect the eluate in 0.5 CV fractions. 20 µl samples of each fraction can be used for SDS- PAGE analysis. The purified *Strep*-tag II fusion protein usually elutes in the 3rd to 5th fractions. If necessary, desthiobiotin and EDTA can be removed via dialysis and/or gel chromatography.

Regeneration of the column

For regeneration, wash the column 3x with 5 CV Buffer R (= Buffer W containing 1 mM HABA). The color change from yellow to red indicates the regeneration process and the intensity of the

red color is an indicator of the column activity status. Remove Buffer R by adding 2 times 4 CV of Buffer W before the next purification run. Store the column at 4 °C overlaid with 2 ml of Buffer W or Buffer R. The purification/regeneration process is essentially the same for columns filled with *Strep-Tactin Sepharose*[®], *MacroPrep*[®] or *Superflow*[®].

Generation of authentic recombinant protein

Remove desthiobiotin by gel chromatography or dialysis after purification of the recombinant protein (with N-terminal *Strep*-tag II and subsequent factor Xa cleavage site). Apply biotinylated factor Xa according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim). After digest, *Strep*-tag II, biotinylated factor Xa, and uncleaved recombinant protein can be separated from the authentic recombinant protein by another *Strep-Tactin* chromatography whereby the authentic recombinant protein is recovered in the flow through fractions.

Short protocol of the *Strep-Tactin* chromatography cycle

1. After the protein extract has entered the *Strep-Tactin* matrix, wash column 5 times with 1 CV (column volume) Buffer W.
2. Elute recombinant protein by the addition of 6 times 0.5 CV Buffer E.
3. Regenerate the column by the addition of 3 times 5 CV Buffer R.
4. Equilibrate the column by the addition of 2 times 4 CV Buffer W prior to the next purification run.
5. Store the column at 4° C overlaid with 2 ml Buffer W or R.

Recommended volumes for working with *Strep-Tactin* columns

Column volume	Protein extract volume*	Washing buffer volume	Elution buffer volume
0.2 ml	0.1 – 2 ml	5 x 0.2 ml	6 x 0.1 ml
1 ml	0.5 – 10 ml	5 x 1 ml	6 x 0.5 ml
5 ml	2.5 – 50 ml	5 x 5 ml	6 x 2.5 ml
10 ml	5 – 100 ml	5 x 10 ml	6 x 5 ml

*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.

Buffer composition

Buffer W (washing buffer) 100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA	Buffer E (elution buffer) 100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA 2.5 mM desthiobiotin	Buffer R (regeneration buffer) 100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA 1 mM HABA (hydroxy-azophenyl- benzoic acid)
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Trouble shooting

Problem: "No or weak binding to Strep-Tactin column"

- *pH is not correct:*
The pH should be > 7.0
- *Strep-tag II is not present:*
Use protease deficient *E. coli* expression strains. Add protease inhibitors during cell lysis.
- *Strep-tag II is not accessible:*
Fuse Strep-tag with the other protein terminus; Use another linker.
- *Strep-tag II is partially accessible:*
Reduce washing volume to 3 column volumes.
- *Strep-Tactin column is inactive:*
Check activity with HABA. Add avidin (Biotin Blocking Buffer) if biotin containing extracts are to be purified. The biotin content of the soluble part of the total *E. coli* cell lysate is about 1 nmol per liter culture (OD 550 = 1.0). Add 2-3 nmol of avidin monomer per nmol of biotin.

Problem: "Contaminating proteins"

Note: The soluble part of the *E. coli* total cell extract contains no proteins beyond the nearly irreversibly binding biotin carboxyl carrier protein (BCCP) which binds significantly to the Strep-Tactin column. Therefore, contaminating proteins are co-purified with the recombinant protein as a result of specific or non-specific interaction.

- *Contaminants are short forms of the tagged protein:*
Use protease deficient *E. coli* expression strains. Add protease inhibitors after cell lysis. Fuse the Strep-tag II with the other protein terminus. Check for the presence of internal translation initiation starts (in case of C-terminal Strep-tag II) or premature termination sites (in case of N-terminal Strep-tag II).
- *Contaminants are covalently linked to the recombinant protein via disulfide bonds:*
Add reducing agents to all buffers for cell lysis and chromatography.
- *Contaminants are non-covalently linked to the recombinant protein:*
Increase ionic strength in all buffers for cell lysis and chromatography (up to 1 M NaCl) or add mild detergents (0.1% Triton X100, 0.1 % Tween, 0.1 % CHAPS).

Problem: "Bubbles in the column"

When the column is taken from the cold storage room to the bench, the temperature difference can cause small bubbles in the column. The reason is, that the cold storage buffer is able to take up more gas than buffers at ambient temperature.

- *To prevent bubbles from developing in the column bed:*
Keep on working in the cold room, use degassed buffers or wash the column immediately with buffers at ambient temperature once the column is removed from the cold.

For research use only

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and Strep-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use. Strep-tag® and Strep-Tactin® are registered trademarks of IBA GmbH.