*Strep*MAB-Classic Purification Protocol

Purification of *Strep*-tag II fusion proteins with immobilized *Strep*-tag II specific monoclonal antibody (*Strep*MAB-Classic affinity purification)

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*Strep*MAB-Classic affinity purification

The *Strep*MAB-Classic monoclonal antibody has been developed to recognize an 8 amino acid sequence (WSHPQFEK) called *Strep*-tag II® in a reversible manner enabling affinity purification combined with mild competitive elution.
The *Strep*MAB-Classic MacroPrep column is particularly useful as an additional purification means after *Strep*-Tactin affinity purification for the isolation of protein complexes for protein:protein interaction studies. It makes use of the same affinity tag albeit with a different purification principle and a different matrix. Thus, possible contaminations (i.e. caused by proteins interacting non-specifically with the Superflow resin or by biotin binding proteins) can be removed to reach extraordinary high purities which may be needed for accurate protein:protein interaction studies.

Comprehensive reviews and scientific publications giving an overview of various *Strep*-tag applications are listed at www.iba-go.com.

Protocol for affinity purification via *Strep*MAB-Classic affinity columns
Perform all operations at a temperature amenable to the stability of your recombinant protein or protein complex (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*MAB-Classic 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 the washing buffer of your choice (e.g. the same buffer in which the recombinant Strep-tag fusion protein had been eluted from the Strep-Tactin column without desthiobiotin). We recommend 100 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA (without EDTA for metalloproteins or protein complexes depending on metal ions) if there are no special requirements with respect to the protein complex. The column cannot run dry under gravity flow.

Adsorption of the *Strep*-tag II fusion protein and column washing
Add the cell extract or the eluate from a *Strep*-Tactin column having a volume between 0.5 and 10 CV to the column (concentrated protein solutions are preferable; if quantification is possible, apply an eluate or cell extract containing between 10 and 30 nmol recombinant *Strep*-tag II fusion protein (complex) 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 or eluate has completely entered the column, wash the column 5 times with 1 CV of washing buffer. Collect the wash fractions (1 CV each) and apply 2 µl of the first (when crude extracts have been used; otherwise, 20 µl shall be used) 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 when crude protein extracts have been used or 20 µl of the eluate from the *Strep*-Tactin column and 20 µl of the flow through when the *Strep*MAB-Classic column has been used as 2nd purification step after *Strep*-Tactin chromatography).

Elution of the recombinant protein
Add 6 times 0.5 CV washing buffer containing 0.5 mM *Strep*-tag peptide (Cat. No. 2-1018-002) and collect the eluate in 0.5 CV fractions. 20 µl samples of each fraction may be used for

SDS-PAGE analysis. The purified *Strep*-tag II fusion protein (complex) usually elutes in the 3rd to 5th fraction. If necessary, peptide and EDTA can be removed via dialysis and/or gel chromatography.
Alternatively, elution can be achieved by using 6 times 0.5 CV 50 mM glycin/HCl pH 3.5 and collect the eluate in 0.5 CV fractions in tubes containing 0.05 CV 1M Tris/HCl pH 8.

Regeneration of the column
To avoid cross-contaminations, re-use of the columns is generally not recommended. To reload the same samples however, columns can be regenerated after elution. To do this, wash the column with 5 CV 50 mM glycin/HCl pH 3.5 after peptide elution. Equilibrate by adding 2 times 4 CV of washing buffer before the next purification cycle. Store the column at 4 °C overlaid with 2 ml of Buffer W.

Recommended volumes for working with *Strep*MAB-Classic 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

*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 100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA

Related products

Cat. No.	Product
2-1003-100	Strep-tag® washing buffer; 100 ml
2-1018-002	Step-tag II Peptide; 1.8 mg
2-1018-009	Step-tag II Peptide; 5x 1.8 mg
2-1507-001	StrepMAB-Classic, purified; 100 µg
2-1526-001	Gravity flow StrepMAB-Classic MacroPrep® column; 1 x 1 ml
2-1526-505	Gravity flow StrepMAB-Classic MacroPrep® column; 5x 0.2 ml

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Trouble shooting

Problem: "No or weak binding to *Strep*MAB-Classic column"

- 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.

Problem: "Contaminating proteins"

- *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.