## FAQ Strep-tag®

Frequently asked questions concerning Strep-tag

### (A) Strep-tag® technology

| Keywords: principle of Strep-tag® technology, size, protease cleavage, spacer, solubility, binding affinity, starterkits, protein-protein-interaction analysis, robotic sample processing systems |

### (B) Cloning and gene expression (see StarGate® System)

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### (C) Strep-tag® purification system

| Keywords: columns, magnetic beads, elution, protein bioactivity, Imidazole, weak binding |

### (D) Purification columns & cartridges

| Keywords: columns, cartridges, adapter, matrix, binding capacity, flow rate, volume capacity, regeneration with HABA, storage, bubbles |

### (E) Alternate buffer conditions

| Keywords: chaotrophic salts, denaturing conditions, reagents compatible with Strep-tag/Strep-Tactin |

### (F) Contaminations

| Keywords: biotinylated proteins, free biotin in medium, BCCP, Biotin-Blocking Buffer, separation of desthiobiotin |

### (G) Regeneration

| Keywords: HABA |

### (H) Detection / Assay

| Keywords: Strep-tag® HRP Detection Kit, Strep-tag® AP Detection Kit, Monoclonal antibodies, immunoprecipitation, pull down assay, in situ-detection, Biacore analysis |
(A) *Strep-tag*® *technology*

(1) **What is the principle of the Strep-tag® technology?**
The Strep-tag® purification system is based on the highly selective and easily controllable interaction between the Strep-tag® II peptide and specially engineered streptavidin called Strep-Tactin®. The tagged protein binds to immobilized Strep-Tactin® during affinity purification. Physiological buffers like PBS in combination with a wide range of additives can be used. After a short washing step, gentle elution of purified recombinant protein is performed by addition of 2.5 mM desthiobiotin in the same buffer. Desthiobiotin is an inexpensive, reversibly binding and stable analog of biotin - the natural ligand of streptavidin. This competitive elution is the second step conferring specificity thus enabling unparalleled purification factors. The system is safe and easy; column regeneration and activity status are visualized by a colour change on the purification column.

(2) **What is the size of the Strep-tag® II?**
Strep-tag® II has eight amino acids (WSHPQFEK) and a molecular weight of 1 kDa.

(3) **Where must the Strep-tag® II be placed?**
It can be attached to the N- or the C-terminus or between two protein domains as a linker. Even the use of Strep-tag® in loop structures is possible.

(4) **What kind of spacer should be used to link a protein with Strep-tag® II?**
We recommend to choose two small, neutral amino acids (like S, A or G). Please try to avoid big, aromatic, charged or structurally potent residues. In our vectors linkers are already included.

(5) **Does the tag increase the solubility?**
Generally small tags do not increase solubility, but in some cases Strep-tag® did.

(6) **What is the binding affinity of a Strep-tag® II protein for Strep-Tactin®?**
$K_D = 1 \mu M$. 

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(7) **Is it possible to use Strep-tag® II with streptavidin or Strep-tag® I with Strep-Tactin®?**

We highly recommend using the *Strep-tag®II:*Strep-Tactin® system, which offers the optimal affinity for purification purposes (sufficient binding and optimal reversibility for mild elution).

<table>
<thead>
<tr>
<th>Interaction</th>
<th><em>K_D</em> Value (µM)</th>
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<tbody>
<tr>
<td>Strep-tag® I: streptavidin</td>
<td>36</td>
</tr>
<tr>
<td>Strep-tag® II: streptavidin</td>
<td>72</td>
</tr>
<tr>
<td>Strep-tag® I: Strep-Tactin®</td>
<td>10 (estimated)</td>
</tr>
<tr>
<td>Strep-tag® II:Strep-Tactin</td>
<td>1</td>
</tr>
</tbody>
</table>

Please note that absolute binding affinity values are strongly dependent on the way of being measured and should not be compared to values determined in different assays. E.g. the *K_D* of the *Strep-tag®I:*streptavidin interaction has been formerly determined to be 0.7 µM (being by a factor of 50 higher than determined with the assay used above (36 µM)) via a titration colorimetry measurement.

(8) **Does the tag bind avidin?**

No. Therefore, avidin can be used in detection assays in order to mask naturally occurring biotinylated proteins which would generate background signals if present in the probe.

For blocking biotinylated proteins in Western blots, we recommend incubating the membrane in Biotin Blocking Buffer [Cat No: 2-0501-002].

More biotin and avidin related FAQs you find in chapter "Contaminations".

(9) **Can the Strep-tag® be cleaved off?**

Yes. However, this is only recommended if absolutely no modification on the recombinant protein is tolerated. Due to its small size and chemically inert nature, *Strep-tag® II* does generally not interfere with the folding or bioactivity of the recombinant protein.

(10) **What are the optimal cleavage conditions to remove the Strep-tag®?**

The cleavage efficiency depends on the accessibility of the cleavage site which is influenced by the proximal 3D structure of the recombinant protein. Therefore, the necessary amount of protease may vary greatly and can not be predicted.
(11) **Do you have a kit for first users?**

Yes, we have several **Starter Kits** [Cat No: 2-1101-000 and 2-1102-001]. The kits come with different columns and include all necessary buffers for expression, purification (binding, washing, elution, regeneration) and detection for the first eight applications in a 100 ml *E. coli* culture scale.

(12) **Which protein serves in the Strep-tag® Starter Kits as a positive control?**

The control protein in the Strep-tag® Starter Kits is azurin (15.1 kDa). In the **Strep/6xHistidine Starter Kit** [Cat No: 2-1117-000] azurin contains two tags (Strep-tag & 6xHistidine-tag) and, therefore, shows a slightly higher molecular weight (16.6 kDa).

(13) **Is it possible to detect protein:protein interactions using Strep-tag® technology?**

Yes, the Strep-tag® is very well suited for the purification of intact protein complexes. Additionally the Twin-Strep-tag® (tandem Strep-tag®) was designed, a tandem arrangement of two Strep-tag®II sequences, with improved performance by increasing purification yields of poorly expressed protein complexes and sustaining elevated detergent concentrations to reduce background.

IBA provides complete solutions for protein:protein-interaction analysis in *E. coli* and mammalia cells with the Twin-Strep-tag® (Cat No: 2-1121-011, 2-1121-012), the One-TAP- [Cat No: 2-1122-011, 2-1122-012], the Two-TAP- [Cat No: 2-1123-011 and 2-1123-012] and the SPINE –Set [Cat No: 2-1124-012], each consisting of a Cloning and a Purification Kit.

(14) **Is the parallel purification of different Strep-tag® proteins possible?**

Yes. We provide **96-well Purification plates** [Cat No: 2-1750-001, 2-1750-011, 2-1750-025, 2-1750-100] for automated, high-throughput purification of Strep-tag® proteins (up to 200 µg Strep-tag® protein per well). The plates are pre-loaded with Strep-Tactin® affinity resin and simply have to be re-hydrated and equilibrated before use. For parallel purification of only a few different Strep-tag® proteins we recommend the fast and easy-to-handle **Strep-Tactin® Spin Columns** [Cat no.: 2-1800-000, 2-1850-010, 2-1850-050].
(15) **Can the Strep-Well HT Purification plate be used with robotic sample processing systems?**

Yes, the Strep-Well HT plate [Cat No: 2-1750-001, 2-1750-011, 2-1750-025, 2-1750-100] is compatible with robotic sample processing systems for example from Tecan, PerkinElmer, Qiagen or Beckman Coulter. The plate can also be used with standard vacuum manifolds for manual sample processing.

(16) **Can the Strep-Well HT Purification plate be used with centrifuges?**

Yes, it is possible to use standard centrifuges. Since protein purification is based on the highly selective binding of Strep-tag® to Strep-Tactin®, the contact time of the lysate with the resin has to be sufficient for complete complex formation. Therefore, it is important not to exceed 700 x g (approx. 2000 rpm in a microfuge) when centrifuging Strep-well spin HT plates for protein binding. Washing steps should be performed at maximum speed.

For very viscous cell lysates, it may be necessary to extend the centrifugation time.

(B) **Cloning and gene expression (see StarGate® System)**

The combinatorial cloning system StarGate® enables the efficient expression of your gene of interest in different hosts like E. coli, yeast, mammalia and insect cells and is an efficient tool to find the most suitable host/tag combination for optimal protein expression.

(C) **Strep-tag® purification system**

(17) **Should columns or magnetic beads be used?**

In general affinity column purification is recommended. Batch purification (with Strep-Tactin® coated magnetic beads or agarose beads) is only recommended if the expression rate of the recombinant protein is above 1 mg per litre E. coli culture. For expression rates lower then 1 mg per litre the Twin-Strep-tag® should be used.
(18) **What degree of purity can be expected?**
Over 95 %. It must be mentioned that potential impurities may result from non-specific interactions of the recombinant protein itself with other proteins leading to lower purity grades. More purity related FAQs you find in chapter "Contaminations".

(19) **How can the protein be eluted?**
Elution is performed by the addition of 2.5 mM desthiobiotin, a derivative of biotin. It is a stable, reversibly binding (columns can be regenerated) low molecular weight substance which does not interfere with the protein or general protein assays.

(20) **Is the protein’s bioactivity preserved?**
Yes. Bioactivity is preserved due to the mild washing and elution conditions by the use of physiological buffers.

(21) **When the protein elutes from the column, is it complexed with desthiobiotin?**
No. Desthiobiotin can be removed via gel filtration or dialysis.

(22) **Is the Strep-tag® system stable in the presence of imidazole?**
Yes, the Strep-tag® system tolerates up to 250 mM imidazole in the protein extract. Therefore, elution fractions from Ni-NTA resins can be directly applied on Strep-Tactin® columns, which is very useful in double-tag purification protocols. In cases where unsatisfying results for Strep-tag® binding from these imidazole containing extracts are observed the imidazole should be removed from the extract.

(23) **What are the possible reasons for no or weak binding to the Strep-Tactin® column?**
- The pH might be incorrect, it should be > 7.0.
- The Strep-tag® is not present or has been cleaved: use protease deficient *E. coli* expression strains or add protease inhibitors during cell lysis to avoid Strep-tag® II degradation; check that the Strep-tag® II is not associated with a portion of the protein that is processed.
If *Strep-tag® II* is not accessible, fuse *Strep-tag® II* with the other protein terminus or use a different linker. If *Strep-tag® II* is only partially accessible, reduce the washing volume to 3 column bed volumes.

(D) **Purification columns & cartridges**

(24) **What is the difference between columns and cartridges?**

Gravity flow columns with *Strep-Tactin® Sepharose, Strep-Tactin® Superflow* or *Strep-Tactin® MacroPrep* resins are designed for gravity flow chromatography. The columns are available in different bed volumes (0,2ml, 1ml, 5ml, 10ml) and package sizes. The cartridges with *Strep-Tactin® Superflow, Strep-Tactin® MacroPrep* or Ni-NTA Superflow resins are designed for automated chromatography workstations with 10-32 connections such as ÄKTA™. The cartridges are pressure resistant for use in FPLC/HPLC, the cover can tolerate 3 bar. The *Strep-Tactin® Superflow, Superflow high capacity* and Ni-NTA Superflow resins are pressure resistant up to 9,6 bar, whereas the *Strep-Tactin® MacroPrep* resin allows 70 bar. The cartridges are also available in different bed volumes 1ml and 5ml.

(25) **Which adapters are necessary using cartridges?**

For HPLC and ÄKTA systems no adapters are needed. For chromatography workstations (or syringes or peristaltic pumps) other than HPLC and ÄKTA, adapters are required [Cat No: 2-1021-001, 2-1022-001, 2-1023-001, 2-1025-001, 2-1026-001]. To connect several cartridges in series to enlarge capacity please use coupling adapters [Cat No: 2-1026-001]. For the use of cartridges with the older Luer-lock system please use syringe adapters.

(26) **What kind of matrix should be used?**

The best support for the purification depends on the recombinant protein and is normally not predictable. For evaluation of the best suited resin for your protein of interest, we are offering *Strep-tag Starter Kit 3C* [Cat No: 2-1102-001]. This comes along with three different gravity flow columns containing *Strep-Tactin®* immobilized to the supports Sepharose®, Superflow® and MacroPrep®.
(27) **What is the maximal binding capacity of each Strep-Tactin® column?**

Depending on protein size between 150 and 500 nmol protein can be purified (up to 3-15 mg of a 20 kDa protein) on a 1ml Strep-Tactin® Superflow high capacity column. Between 50 and 100 nmol protein can be purified on a 1 ml column of MacroPrep, Sepharose or Superflow.

(28) **What is the recommended linear flow rate* for Strep-tag® purification?**

<table>
<thead>
<tr>
<th>Sepharose</th>
<th>Superflow</th>
<th>MacroPrep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity flow</td>
<td>Up to 300 cm/h</td>
<td>Up to 300 cm/h</td>
</tr>
</tbody>
</table>

* Volumetric flow rate (ml/min) = Linear flow rate (cm/h) divided by 60 and multiplied by the column cross sectional area (cm²). For more information click here.

(29) **Which volumes should be used?**

<table>
<thead>
<tr>
<th>Column bed volume:</th>
<th>Protein extract volume:</th>
<th>Washing buffer volume:</th>
<th>Elution buffer volume:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ml</td>
<td>0.1 – 2 ml</td>
<td>5 x 0.2 ml</td>
<td>6 x 0.1 ml</td>
</tr>
<tr>
<td>1 ml</td>
<td>0.5 – 10 ml</td>
<td>5 x 1 ml</td>
<td>6 x 0.5 ml</td>
</tr>
<tr>
<td>5 ml</td>
<td>2.5 – 50 ml</td>
<td>5 x 5 ml</td>
<td>6 x 2.5 ml</td>
</tr>
<tr>
<td>10 ml</td>
<td>5 – 100 ml</td>
<td>5 x 10 ml</td>
<td>6 x 5 ml</td>
</tr>
</tbody>
</table>

(30) **How often can the column be re-used?**

3 - 5 times, without loss in performance.

(31) **How should the columns be stored?**

The columns should be stored at 4 °C, overlayed with buffer W. For shorter periods (only a few days) buffer R can be used.

(32) **How can air bubbles in the column be avoided?**

When the column is taken from the cold storage room to the bench, the increased temperatures can cause small air bubbles in the column. The reason is that buffers are able to take up more gas in the cold than room temperature. To avoid bubbles keep on working in the cold room, use degased buffers or wash the column with buffers equilibrated at room temperature immediately after the column is transferred from the cold room to the bench.
Alternate buffer conditions

(33) Can the column be treated with chaotropic salts?
Yes. 1 column volume of 6 M guanidine/HCl can be applied for removing aggregates which may have formed after several purification runs. Do not store the column in the presence of guanidine. Restore immediately native buffer conditions after guanidine treatment by washing with, for example, Tris buffer at pH 8.0 (e.g. Buffer W).

(34) Is it possible to purify under denaturing conditions (6 M urea, 6 M guanidinium chloride)?
No, the tag has been developed to purify proteins under physiological conditions in order to obtain bioactive proteins.

(35) Can detergents or other buffer systems be used?
Yes. High salt, reducing reagents like mercaptoethanol, chelating reagents, and detergents are allowed but not necessary. However, the pH has to be above pH 7.0.

(36) Which reagents are compatible with the Strep-tag®/Strep-Tactin® interaction?
To get a list of reagents compatible with the Strep-tag®/ Strep-Tactin® interaction please click here.

Contaminations

(37) Is the presence of biotinylated proteins in the host organism in case of proteins expressed in the cytoplasm a problem?
No. Generally, the cell internal content of biotinylated proteins and free biotin is rather low and not a threat for significant inactivation of the Strep-Tactin® resin. Therefore, no avidin has necessarily to be added to the extract when purification is intended. See the following examples for the cell internal biotin content of some organisms: An E. coli extract derived from a 1 l culture with OD550 = 1 has a total biotin content of around 1,75 nmol only (biotin capacity ≈ 350 nmol/ml sedimented resin). The total internal biotin content in CHO
cell extract is not measurable indeed. And the extract of $1 \times 10^8$ HEK-293 cells contains only 0,5µg Biotin. For detailed information please click here.

(38) **Is the presence of free biotin in the medium (in case of proteins secreted to the medium) a problem (eukaryotic expression)?**

Free Biotin inactivates *Strep*-Tactin® resins (biotin capacity $\approx 350$ nmol/ml sedimented resin) and has to be removed or masked prior to affinity chromatography. This is mostly relevant when cell culture supernatant, containing secreted recombinant protein, is directly subjected to *Strep*-Tactin® affinity chromatography because some media for insect cells or mammalian cells contain significant amounts of biotin. To get an overview of Biotin contents in cell culture media and Biotin free media, please download this pdf.

The simplest way to get rid of the biotin problem for purification of secreted eukaryotic proteins is irreversible masking by the addition of avidin. IBA provides different products for biotin blocking, depending on what application it is used for.

For purification from **cell culture supernatants** with cell culture media containing Biotin we recommend our ready-to-use **BioLock Biotin Blocking Solution** [Cat No: 2-0205-050] with an activity of $>70$U/ml.

(39) **How to avoid binding of unspecific biotinylated proteins in protein interaction analysis experiments?**

For **protein interaction** experiments even the cell internal Biotin content (especially the biotinylated proteins) is of importance because they lead to unspecific (false positive) binding of these biotinylated proteins to *Strep*-Tactin®. This can be avoided by adding our **high grade lyophilized Avidin** powder [Cat No: 2-0204-015] with an activity of 11U/mg.

Add 1U avidin per µg contaminating biotin.

(40) **Does the biotinylated E. coli protein BCCP interfere with the binding of *Strep-tag*® proteins to the resin?**
BCCP usually does not harm during purification, since the intracellular concentration of this protein is quite low. The binding of BCCP to the Strep-Tactin® resin can be avoided by adding avidin to the cell lysate before chromatography (20µg/litre of an E. coli culture at OD600=1).

(41) **How can additional bands from biotinylated proteins on Western blots be avoided?**

For blocking biotinylated proteins in Western blots, we offer a Biotin Blocking Buffer containing avidin [Cat No: 2-0501-002]. Use a dilution of 1:1000 in standard Western blot blocking reagent prior to detecting Strep-tag® proteins with Strep-Tactin® conjugates. Alternatively, monoclonal antibodies against Strep-tag® [Cat No: 2-1507-001, 2-1509-001] can be used, which do not recognize biotin.

(42) **How can desthiobiotin be separated from the Strep-tag® protein after elution?**

Either use gel filtration or dialyse the elution fractions against your buffer of choice. Usually this is not necessary, though.

(43) **How can non-specifically bound proteins be removed?**

If the contaminants are covalently linked to the recombinant protein via disulfide bonds, add reducing agents to all buffers for cell lysis and chromatography. In case of non-covalently linked contaminants, increase the ionic strength in all buffers for cell lysis and chromatography (up to 1 M NaCl) or add 0.1 % mild detergents (Triton X-100, Tween 20, CHAPS, etc).

(44) **Apart from unspecific binding: What are the possible reasons for protein contamination?**

Co-purifying proteins may be proteins that interact via non-specific interactions (impurities) or via specific interactions (functional protein complex) with the recombinant Strep®-tag fusion protein.

(45) **How can the level of unspecific binding to magnetic beads be reduced?**
To avoid unspecific binding add 0.05% Tween 20 to the washing buffer, use high salt concentrations (0.3 M NaCl in 0.05 M phosphate buffer) or lower the pH to 7.

(G) Regeneration

(46) Why is the colour of the MacroPrep® Strep-Tactin® column not intense red after regeneration with HABA?

MacroPrep is not as transparent as Sepharose® or Superflow®. Thus, the colour shift is to some kind of pink only. For further information and pictures of the resin regeneration with HABA please download this manual.

(47) How can the Strep-Tactin® matrix be regenerated?

If desthiobiotin is used for elution, the Strep-Tactin® matrix can be regenerated with an azo dye (HABA) which, when applied in excess, displaces desthiobiotin. The dye is yellow in solution and shifts to red when bound by Strep-Tactin® which allows the visual control of the regeneration process and the functional status of the column. As long as a colour gradient between the top and the bottom of the column is visible, it is not fully regenerated.

Regeneration is not possible, if biotin has been used for elution (Strep-Tactin® Spin Columns, Strep-Well HT Purification Plates, MagStrep (Strep-Tactin® coated Magnetic Beads), Twin-Strep-tag® - protein:protein interaction analysis) since biotin binds nearly irreversibly to Strep-Tactin®.

(48) What is the mechanism for HABA displacing desthiobiotin from the Strep-Tactin® column?

HABA binds to the same Strep-Tactin® binding pocket as Strep-tag® (or biotin) does, but with very low affinity only. Nevertheless, it accelerates desthiobiotin removal because it is applied in excess. That means each time when a Strep-Tactin®: desthiobiotin complex dissociates on the column, the resulting free Strep-Tactin® binding site may be occupied by HABA or desthiobiotin in competition. As HABA is applied in excess, desthiobiotin has only a reduced chance to find a free binding pocket, which finally results in accelerated removal of desthiobiotin from the column.
(49) Can the Strep-Tactin® matrix be treated with NaOH?
Yes, it is also possible to regenerate the columns with 0.5M NaOH. Add 5 column volumes 0.5M NaOH. After the flow through add directly buffer W, pH 8.0. Please do not incubate with NaOH for a longer period. We recommend the use of NaOH for cleaning the column from unspecific bound or precipitated protein.
For the removal of desthiobiotin please use buffer R with HABA. Buffer R is the specific and smooth cleaning buffer and will not damage the Strep-Tactin® resin whereas the longer use of NaOH will damage the resin following by the loss of binding capacity.

(H) Detection / Assay

(50) How can the tag be detected?
For fast detection we offer Strep-Tactin® conjugated to HRP or AP. The Strep-tag® HRP Detection Kit [Cat No: 2-1502-000] and the Strep-tag® AP Detection Kit [Cat No: 2-1503-000] provide the necessary tools to detect Strep-tag®II fusion proteins in Western blots. These ready-to-use systems include all buffers and reagents for blocking, washing and the chromogenic reaction. For high specificity the monoclonal antibody against Strep-tag®II can be used [Cat No: 2-1507-001, 2-1509-001]. It is available affinity purified and unlabelled or affinity purified and labelled with HRP.

(51) Which detection procedure (ECL or chromogenic) do you recommend for HRP Strep-Tactin® conjugates?
Both, chemiluminescence as well as chromogenic detection, are possible. Please note that the application protocols for these procedures differ. The protocols are described in the "Western blot protocol" which is also available as pdf.

(52) What are the KD-values of the monoclonal antibodies?
K_D StrepMAB-Classic = approximately 1 µM.
K₀ StrepMAB-Immo: approximately 1 pM.

These antibodies are monoclonal mouse antibodies, type IgG1.

(53) **Do the StrepMAB antibodies also work in immunoprecipitation and pull down assays?**

Yes, several applications are possible. We recommend to use the Twin-Strep-tag® (Strep-tag® II is also possible) for the bait protein and to precipitate with Strep-Tactin® Superflow or Strep-Tactin® magnetic beads using biotin for the elution step. Alternatively, StrepMAB-Classic MacroPrep can be used for precipitation using the Strep-tag® peptide for the elution step. Please note, if you have to use classical immunoprecipitation without an elution step, we recommend StrepMAB-Immo MacroPrep for immunoprecipitation because of its nearly irreversible binding to the Twin-Strep-tag® or Strep-tag® II.

(54) **Can a tagged protein be detected in situ?**

Yes. It can be detected via EM or FM with streptavidin which is labelled with colloidal gold or FITC.

(55) **Has the Strep-tag® technology been tested in Biacore analysis?**

Yes. StrepMAB-Immo, high-affinity Strep-tag® II specific monoclonal antibody [Cat No: 2-1517-001], is optimal for capturing SerAla-Strep-tag® II fusion proteins on solid phases such as Biacore chips or microplates.