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(A) *Strep-tag® technology*

(1) *What is the principle of the Strep-tag® technology?*
The *Strep*-tag® purification system [http://www.iba-go.de/prottools/index.php?prot_p_puri.html] 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 µM.

(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).

K_D (*Strep*-tag I:streptavidin) = 36 µM
K_D (*Strep*-tag II:streptavidin) = 72 µM
K_D (*Strep*-tag I:*Strep*-Tactin) = 10 µM (estimated)
K_D (*Strep*-tag II:*Strep*-Tactin) = 1 µM

Please notice 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 [http://www.iba-go.de/prottools/index.php?prot_p_star.html] [Cat.-no.: 2-1101-000, 2-1102-001, 2-1103-001, 2-1104-000, 2-1115-000, 2-1116-000]. The kits come with different amounts of columns or cartridges 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* [http://www.iba-go.de/prottools/index.php?prot_p_his2.html] [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 One 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 One-STrEP- [Cat.-no.: 2-1121-001], the One-TAP- [Cat.-no.: 2-1122-001], the Two-TAP- [Cat.-no.: 2-1123-000] and the SPINE -Set [Cat.-no.: 2-1124-000], each consisting of a Cloning and a Purification Kit. [http://www.iba-go.com/prottools/index.php?prot_p_ppi-intro.html]

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

Yes. We provide 96-well plates [http://www.iba-go.de/prottools/index.php?prot_p_htp.html&96prot] [Cat.-no.: 2-1700-000, 2-1725-010, 2-1705-010, 2-1706-010, 2-1707-010] 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 [http://www.iba-go.de/prottools/index.php?prot_p_spin.html] [Cat-no.: 2-1800-000, 2-1850-050].

(15) *Can the Strep-Well HT Purification plate be used with robotic sample processing systems?*

Yes, the plate [http://www.iba-go.de/prottools/index.php?prot_p_htp.html&96prot] [Cat.-no.: 2-1700-000, 2-1725-010, 2-1705-010, 2-1706-010, 2-1707-010] is compatible with robotic sample processing systems, such as the Freedom EVO® 200 (Tecan), the MultiPROBE® II/Evolution P^3 (PerkinElmer), the BioRobot workstations (Qiagen) and the Biomek FX^P/NX^P/3000 robots (Beckman Coulter). The plate can also be used with standard vacuum manifolds for manual sample processing.

(B) *Cloning and gene expression*

Please refer to the StarGate® section [http://www.iba-go.de/naps/naps_fr04_01.html].

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*

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

Generally affinity column purification is recommended. Batch purification (with *Strep*-Tactin® coated magnetic beads) is only recommended if the expression rate of the recombinant protein is above 1 mg per litre *E. coli*

culture. Lower expression rates are possible using the One-StrEP-tag or the *Strep*-Tactin Superflow high capacity matrix.

(17) *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*".

(18) *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.

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

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

(21) *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.

(D) *Purification columns & cartridges*

(22) *What is the difference between columns and cartridges?*
Gravity flow columns [http://www.iba-go.de/prottools/prot_p_cofo.html] with *Strep*-Tactin Sepharose [Cat.-no.: 2-1202-001], *Strep*-Tactin Superflow [Cat.-no.: 2-1207-001] or *Strep*-Tactin MacroPrep [Cat.-no.: 2-1506-001] resins are designed for gravity flow chromatography.
Pre-packed columns with *Strep*-Tactin POROS resin [Cat.-no.: 2-1203-017, 2-1205-017] are designed for high linear flow rates.

The new H-PR cartridges [http://www.iba-go.de/prottools/prot_p_cofo.html#cart] ("highly pressure-resistant") with *Strep*-Tactin Superflow [Cat.-no.: 2-1231-001, 2-1232-001], *Strep*-Tactin MacroPrep [Cat.-no.: 2-1532-001, 2-1511-001] or Ni-NTA Superflow [Cat.-no.: 2-3231-001, 2-3232-001] resins are designed for automated chromatography workstations with 10-32 connections such as ÄKTA™. The H-PR cartridges are highly pressure resistant for use in FPLC/HPLC, can tolerate 20 bar and can also be used with a flow restrictor.
Our older version of Luer-lock cartridges, which were also designed for FPLC and HPLC, is about to be discontinued.

(23) *Which adapters are necessary using H-PR cartridges?*
For HPLC and ÄKTA systems no adapters are needed. For chromatography workstations (or syringes or peristaltic pumps) other than HPLC and ÄKTA, adapters [http://www.iba-go.de/prottools/index.php?prot_p_cofo.html#adapters] are required [Cat.-no.: 2-1022-001, 2-1023-001, 2-1025-001, 2-1026-001]. To connect several H-PR cartrigdes in series to enlarge capacity please use coupling adapters [Cat.-no.: 2-1026-001].
For the use of H-PR cartridges with the older Luer-lock system please use syringe adapters [Cat.-no.: 2-1021-001].

(24) *What kind of matrix should be used?*
The best support for the purification depends on the recombinant protein and is normally not predictable. For rapid optimization of your purification results, we are offering two column evaluation sets [http://www.iba-go.de/prottools/index.php?prot_p_evk.html] [Cat.-no.: 2-1001-001, 2-1001-011] with *Strep*-Tactin immobilized to the supports Sepharose®, Superflow® or MacroPrep® [http://www.iba-go.de/prottools/index.php?prot_p_resin.html].

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

Sepharose	Superflow	MacroPrep	POROS
gravity flow	Up to 300cm/h	Up to 300 cm/h	300 - 500 cm/h

* Volumetric flow rate (ml/min) = Linear flow rate (cm/h) divided by 60 and multiplied by the column cross sectional area (cm^2)

(26) *Which volumes should be used?*

Column bed 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

(27) *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.

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

4 °C, overlayed with buffer W or, for shorter periods, with buffer R.

(E) *Alternate buffer conditions*

(29) *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).

(30) *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.

(31) *Which reagents are compatible with the Strep-tag®/Strep-Tactin® interaction?*

Reagent	Concentration
Reduction Agents	

DTT	50 mM
β-mercaptoethanol	50 mM
Non-Ionic Detergents	
C_8E_4; Octyltetraoxyethylene	max. 0.88 %
$C_{10}E_5$; Decylpentaoxyethylene	0.12 %
$C_{10}E_6$	0.03 %
$C_{12}E_8$	0.005 %
$C_{12}E_9$; Dodecyl nonaoxyethylene (Thesit)	0.023 %
DM; Decyl-β-D-maltoside	0.35 %
LM; N-dodecyl-β-D-maltoside	0.007 %
NG; N-nonyl-β-D-glucopyranoside	0.2 %
OG; N-octyl-β-D-glucopyranoside	2.34 %
TX; Triton X-100	2 %
Tween 20	2 %
Ionic Detergents	
N-lauryl-sarcosine	2 %
8-HESO;N-octyl-2-hydroxy-ethylsulfoxide	1.32 %
SDS; Sodium-N-dodecyl sulfate	0.1 %
Zwitter-Ionic Detergents	
CHAPS	0.1 %
DDAO; N-decyl-N,N-dimethylamine-N-oxide	0.034 %
LDAO; N-dodecyl-N,N-dimethylamine-N-oxide	0.13 %
Others	
Ammonium sulfate $(NH_4)_2SO_4$	2 M
$CaCl_2$	max. 1 M
EDTA	50 mM
Ethanol	10 %
Guanidine	max. 1 M
Glycerol	max. 25 %
Imidazole	max. 250 mM
$MgCl_2$	1 M
NaCl	5 M
Urea	max. 1 M

Note: These reagents have been successfully tested for the purification of e.g. GAPDH-*Strep*-tag with concentrations up to those mentioned. For reagents not marked with "max." higher concentrations may be possible, though. Since binding depends on the sterical accessibility of *Strep*-tag in the context of the particular protein the possible concentration may deviate from the given value for other proteins.

(F) *Contaminations*

(32) *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.

Example: An *E. coli* extract derived from a 1 l culture with OD_{550} = 1 has a total biotin content of around 1 nmol only (biotin capacity ≅ 350 nmol/ml sedimented resin).

(33) *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 ≅ 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 (see table below). The simplest way to get rid of the biotin problem for purification of secreted eukaryotic proteins is irreversible masking by the addition of avidin [Cat.-no.: 2-0204-050] using 120 mg avidin per mg contaminating biotin. This procedure is recommendable at high expression levels.

Biotin contents of standard mammalian cell culture media (1 mg biotin corresponds to 4 µmol):

Medium	**D-Biotin [mg/l]**	**Citation**
BME (Eagle)	1	Eagle H. (1965), Proc. Soc. Exp. Med. 89, 362
CMRL 1066	0.01	Parker, R.C., et al. (1957) Special Publications, N.Y. Academy of Sciences, 5, 303
DMEM	-	Dulbecco, R. Freeman, G. (1959) Virology 8, 396. Smith, J.D., Vogt, M. and Dulbecco, R. (1960) Virology 12, 185
DMEM/F-12	0.035	
Hams F10	0.024	Ham, R.G. (1963), Exp. Cell Res., 29, 515

Hams F12	0.007	Ham, R.G. (1965), Proc. Nat. Acad, Sci., 53, 288
Fischer's	0.01	Fischer, G.A. and Sartorelli, A.S. (1964), Methods in Med. Res. 10.
Iscove's (IMDM)	0.013	
Leibovitz's L-15	-	Leibovitz, A. (1963) Am. J. Hyg. 78, 173
McCoys 5A	0.2	
MCDB 131	0.0073	
Medium 199	0.01	Morgan, Morton and Parker (1950) Proc. Soc. Exp. Biol. Med., 73, 1
α MEM	0.1	
NTCC 109/135	0.025	
RPMI 1640	0.2	Moore, G.E., Gerner, R.E. and Franklin, H.A. (1967) A.M.A. 199, 519
Waymouth's MB 752/1	0.02	
Williams' E	0.5	Williams, G.M. and Gunn, J.M. (1974) Exp. Cell. Res., 89, 39

Biotin contents of standard insect cell culture media (1 mg biotin corresponds to 4 µmol):

Medium	**D-Biotin [mg/l]**	**Citation**
Grace's	0.01	Grace, T.C. (1962) Nature 195, 788
Schneider's	-	Schneider, I. (1964), Exp. Zool. 156, 1 , 91
SF900II from Invitrogen	0.1	determined by IBA
SF3 from PromoCell	0.1	determined by IBA
ExCell 401 from JRH Biosciences	0.1	determined by IBA
Express Five from Gibco/Invitrogen	0.15	determined by IBA
Graces from Gibco/Invitrogen	-	determined by IBA

Insect Xpress from Cambrex	0.1	determined by IBA

(34) *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 [http://www.iba-go.de/prottools/prot_p_bur.html#biotin] 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.

(35) *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.

(G) *Regeneration*

(36) *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, Mag*Strep* (Strep-Tactin coated Magnetic Beads), One-StrEP protein:protein interaction analysis) since biotin binds nearly irreversible to *Strep*-Tactin.

(37) *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.

(H) *Detection / Assay*

(38) *How can the tag be detected?*
For fast detection we offer *Strep*-Tactin conjugated to HRP or AP. The *Strep-tag HRP Detection Kit* [http://www.iba-go.de/prottools/index.php?prot_p_hrp.html] [Cat.-no.: 2-1502-000] and the *Strep-tag AP Detection Kit* [http://www.iba-go.de/prottools/index.php?prot_p_ap.html] [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 [http://www.iba-go.de/prottools/index.php?prot_p_poly.html] 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.

(39) *Which detection procedure (ECL or chromogenic) do you recommend for HRP Strep-Tactin® conjugates?*
Both, chemiluminescene 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 on the IBA download/tech info site [http://www.iba-go.de/download.html].

(40) *What are the K_D-values of the monoclonal antibodies?*
K_D *Strep*MAB-Classic = approximately 1 µM.
K_D *Strep*MAB-Immo: approximately 1 pM.
These antibodies are monoclonal mouse antibodies, type IgG1.

(41) *Do the StrepMAB antibodies also work in immunoprecipitation and pull down assays?*
Yes, several applications are possible. We recommend to use the One-STrEP-tag [http://www.iba-go.de/prottools/prot_p_one.html] (*Strep*-tag II is also possible) for the bait protein and to precipitate with *Strep*-Tactin Superflow

[http://www.iba-go.de/prottools/prot_p_sfl.html] or *Strep*-Tactin magnetic beads [http://www.iba-go.de/prottools/prot_p_mag.html] using biotin for the elution step. Alternatively, *Strep*MAB-Classic MacroPrep [http://www.iba-go.de/prottools/prot_p_one.html#classic] can be used for precipitation using the *Strep*-tag peptide [http://www.iba-go.de/prottools/prot_p_one.html#peptide] for the elution step. Please note, if you have to use classical immunoprecipitation without an elution step, we recommend *Strep*MAB-Immo MacroPrep [http://www.iba-go.de/prottools/prot_p_one.html#immo_col] for immunoprecipitation because of its nearly irreversible binding to the One-StrEP-tag or *Strep*-tag II.

(42) *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.

(43) *Has the Strep-tag® technology been tested in Biacore analysis?*
Yes. *Strep*MAB-Immo [http://www.iba-go.de/prottools/prot_p_poly.html#strepMAB], 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.