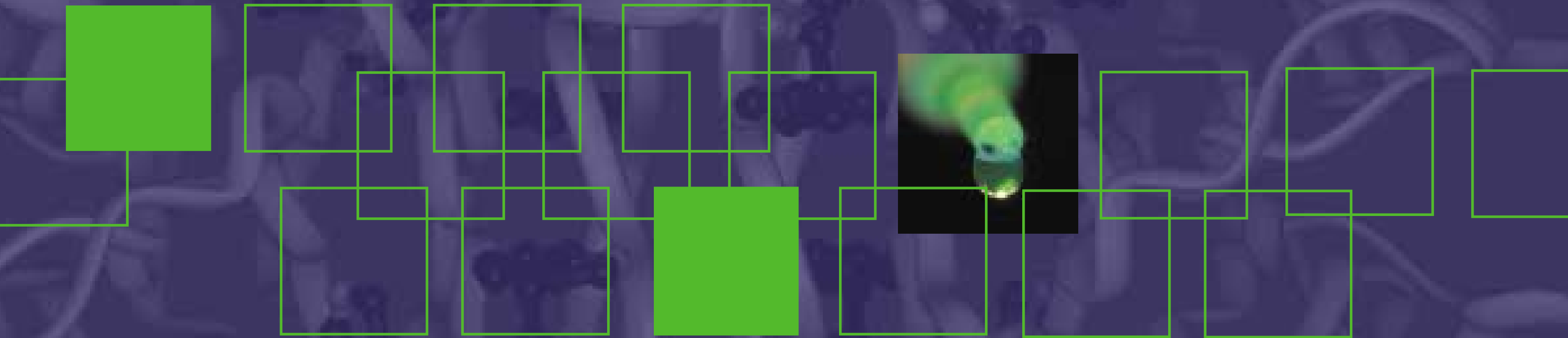
Tools for high-throughput protein expression & purification

Strep-Well HT purification plates

Strep-tag vectors 6xHis-tag vectors

Strep-tag technology

Tools for protein expression & purification



Strep-tag vectors 6xHis-tag vectors

Strep-tag technology

6xHis-tag & Ni-NTA technology

High performance protein expression & purification

Tools for high-throughput protein expression & purification

Strep-tag® technology 11

High performance protein expression and purification

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The optimal partner for *Strep*-tag in double tag proteins

Vectors 41

With *Strep*-tag and/or 6xHis-tag

Tools for high-throughput protein expression & purification 51

Strep-Well HT purification plates

Strep-Well HT purification plates

- Simultaneous purification of 96 *Strep*-tag® proteins
- Up to 200 µg highly pure *Strep*-tag protein per well
- Convenient ready-to-use format
- Pre-filled with immobilized *Strep*-Tactin®
- Time-saving
- Compatible with vacuum manifolds and robotic systems

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Details see pages 51 ff.

Strep-tag® technology

High-performance protein expression and purification

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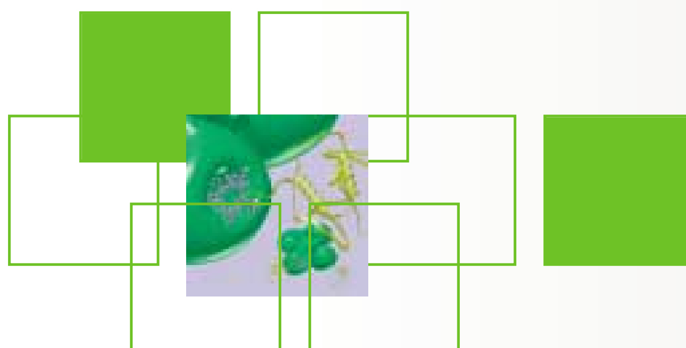


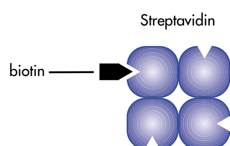
Using the *Strep*-tag system, we succeeded for the first time in obtaining active enzyme" (Wendt *et al*, 2000)

Introduction

The *Strep*-tag® story

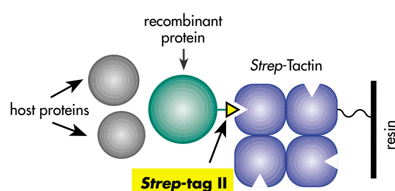
- Undisturbing short tag
- Rapid one-step purification under physiological conditions
- Unsurpassed purity and bioactivity

Being one of the foremost providers of expression cloning and protein purification technology in the post genomic era, IBA has developed a universal platform called ***Strep*-tag®**. This platform for the **rapid and cost effective as well as versatile production and use of recombinant proteins** was developed in close cooperation with Prof. Dr. Arne Skerra, TU Munich. In addition to our large product portfolio around *Strep*-tag (see page 9 ff.), we also provide exclusive custom protein production using this technology (see page 58 ff.).



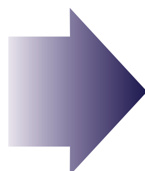
The basis for the development of the *Strep*-tag principle was the well known binding of biotin to streptavidin. To take advantage of this strong interaction in protein purification applications we found it desirable to have a peptide that is capable of binding to the biotin binding pocket of streptavidin when fused to recombinant proteins. This peptide was supposed to serve as purification tag. Finally, we succeeded in engineering a short sequence consisting of only 8 amino acids (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) and named it *Strep*-tag II.

To optimize binding properties, also streptavidin has been engineered to obtain *Strep*-Tactin. Thus, the optimal binding partners have been found: **The *Strep*-tag / *Strep*-Tactin system is now one of the most widely used affinity chromatography systems.**

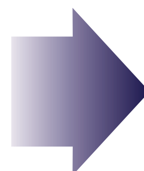


Strep-Tactin coupled to various matrices allows affinity purification of *Strep*-tag fusion proteins under physiological conditions. In contrast to other tags, these mild purification parameters preserve bioactivity of the protein and may yield over 99% purity after a single chromatographical step (see page 22).

Using a simple and universal cloning strategy provided by the IBA expression vectors, *Strep*-tag can be genetically fused to a protein's N- or C-terminal end (see page 41 ff.). In addition, the *Strep*-tag can also be used in the context of other expression systems (incl. mammalian systems). Detection systems based on *Strep*-Tactin



binding
GFP *Strep*-tag protein binds to *Strep*-Tactin



elution
with desthiobiotin

regeneration with color control
beginning of desthiobiotin removal

Please note, that the resin used in this example is *Strep*-Tactin Sepharose. Other resins, such as *Strep*-Tactin MacroPrep, may not show such a clear color change (see page 20).

directly conjugated to reporter enzymes or antibodies are fast, selective and sensitive (see page 29). Furthermore, the specific interaction enables the selective and oriented immobilization of the target protein on *Strep*-Tactin coated surfaces. Thus, microplates coated with *Strep*-Tactin are a general platform for straightforward assays of tagged target proteins, in particular for high throughput screening assays (see page 33).

The tag

- Just 8 amino acids
- Balanced amino acid composition - generally no effect on protein structure or activity
- Highly selective and easily controllable binding properties
- C- or N-terminal fusion
- Removal not required

The technology

- Efficient and versatile bacterial expression vectors with standardized cloning strategy
- Affinity chromatography under physiological conditions
- Column regeneration and activity status is visualized by color change (see below)
- Over 99% purity can be achieved

The proteins

Strep-tag is the method of choice for:

- metalloproteins
- membrane proteins
- sensitive protein complexes with multiple subunits
- and any other protein (examples see page 15)!

Metalloenzymes

E. coli alkaline phosphatase

Reference:

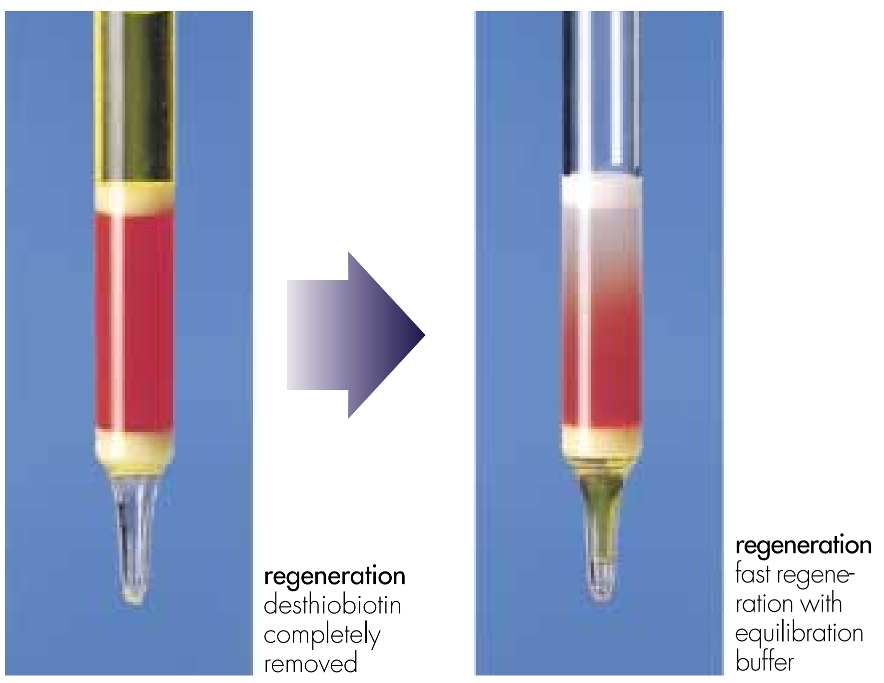
Hengsakul M, Cass AEG, 1997: J. Mol. Biol. 266: 621-632. Alkaline phosphatase-*Strep*-tag fusion protein binding to streptavidin: Resonant mirror studies.

Heterodimeric proteins

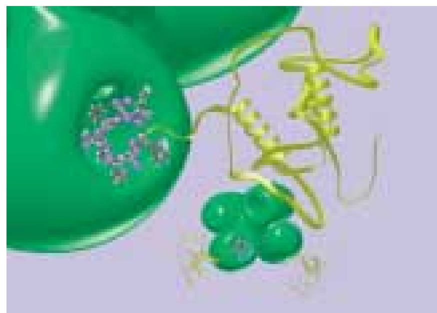
H. pylori urease

Reference:

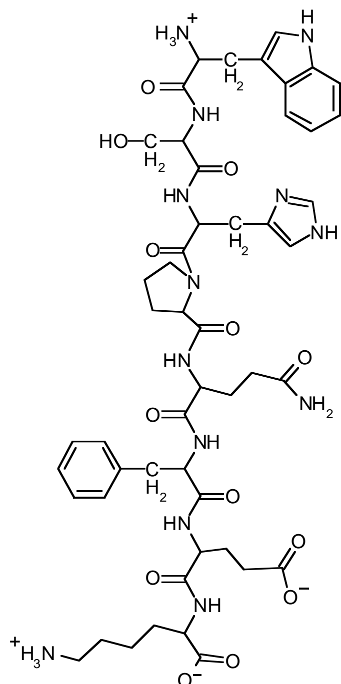
Schmidt TGM, Skerra A, 2000: in Alberghina L (Ed.), Protein Engineering in Industrial Biotechnlogy, Harwood Academic Publishers, ISBN: 90-5702-412-8, pp. 41-61. Protein Engineering for Affinity Purification: the *Strep*-tag.



Purification of a GFP-*Strep*-tag II fusion protein, which has been overexpressed in *E. coli* (see also page 17). Pictures left to right: 1, specific binding of GFP-*Strep*-tag II fusion protein to *Strep*-Tactin Sepharose column while unspecific proteins are rapidly washed away with small amounts of physiological buffer; 2, *Strep*-tag protein is eluted due to addition of the specific competitor "desthiobiotin"; 3 to 5, column regeneration: desthiobiotin is displaced by the yellow solution HABA, which turns red once complexed with *Strep*-Tactin. HABA is then removed by washing buffer and the column can be re-used. For desthiobiotin and buffer order information see page 28.



A *Strep*-tag protein is binding to a *Strep*-Tactin tetramer.



Strep-tag II
NH_2-WSHPQFEK-COOH

Principle and properties

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 binding affinity of *Strep*-tag II to *Strep*-Tactin is nearly 100 times higher** than to streptavidin. 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 (see Table 1, page 20). After a short washing step, gentle elution of purified recombinant protein is performed by addition of desthiobiotin (2.5 mM) 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 to use**; column regeneration and activity status are visualized by a color change on the purification column (see pages 12, 17).
Convenient detection of *Strep*-tag fusion proteins can be performed in Western blots, ELISA, electron or fluorescence microscopy. Appropriate *Strep*-Tactin enzyme conjugates and monoclonal antibodies against *Strep*-tag II are available (see pages 29-32).

Strep-tag® II

The short peptide tag (8 amino acids) has negligible effect on the recombinant protein due to its chemically balanced amino acid composition (WSHPQFEK). The tag can be placed at the C- or N-terminus. A two amino acid spacer between the protein and the tag is recommended to ensure accessibility of the tag. Generally, it does **not interfere with folding or bioactivity**, does not react with heavy metal ion buffer impurities, has no ion exchange properties and does not induce protein aggregation. Thus, there is **no need for removing the tag**.

Strep-Tactin®

Strep-Tactin is a streptavidin derivative which is **one of the most stable proteins known**. Streptavidin is stable to treatment with 8 M urea or guanidine, 0.5 M NaOH as well as 50 % formamide (t = 1 h; T = 37 °C). Proteases (proteinase K, pepsin, papain, subtilisin, thermolysin, elastase) do not cleave streptavidin during a 2 h incubation at a 1:50 w/w ratio and 37 °C. In the presence of SDS streptavidin begins to break up into monomers only at temperatures above 60 °C. As far as tested, we have been able to confirm these extraordinary properties for *Strep*-Tactin, thus **enabling long-lasting affinity columns** which can be re-used 3-5 times. Furthermore, the neutral pI of *Strep*-Tactin minimizes non-specific protein or nucleic acid binding*.

Benefits

- Purification of bioactive recombinant proteins
- Physiological purification using desthiobiotin elution
- Protein aggregation is avoided
- Broad range of detergents, chelators, salt or redox conditions allowed
- Avoids interaction with heavy metal ions which are toxic and may catalyze protein oxidation

*For reagents compatible with the *Strep*-tag/*Strep*-Tactin interaction refer to table 1, page 20.

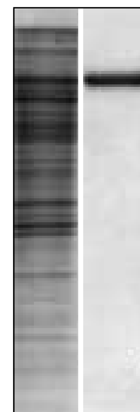
Examples

of proteins successfully expressed as *Strep*-tag® fusion proteins

***Strep*-tag fusion protein**	***Strep*-tag application (References see page 35)**
V_H domain of the lysozyme-binding D1.3 F_v fragment (mouse hybridoma)	Purification of the intact F_v fragment Detection of the F_v fragment via ELISA and Western blotting
V_L domain of the D1.3 F_v fragment (mouse hybridoma)	Purification of the intact F_v fragment Detection of the F_v fragment via ELISA and Western blotting Capture of the F_v:antigen complex
Azurin *(P. aeruginosa)*	Purification of the functional metalloprotein
Cytochrome b_{562} *(E. coli)*	Purification of the functional heme protein
Cystatin (chicken)	Purification of the functional protein
Retinol-binding protein (pig)	Purification of the functional protein
Bilin-binding protein and mutants *(Pieris brassicae)*	Purification of the functional protein (47) ELISA applications (47)
Fragment of the growth-promoting activity receptor A (chicken)	Purification of the functional protein fragment
Fragment of the ciliary neurotrophic factor receptor (chicken)	Purification of the functional protein fragment
V_H domain of the cytochrome C oxidase-binding 7E2 F_v fragment (mouse hybridoma)	Purification of the intact F_v fragment (44) Capture of the intact cytochrome C oxidase (4 subunits) (19) Detection of the F_v fragment via immunoelectron microscopy (53) and immunofluorescence microscopy (56)
V_H domains of the ubiquinol:cytochrome Coxidoreductase-binding 7D3 and 2D6 F_v fragments (mouse hybridomas)	Purification of the intact F_v fragment (53) Capture of the intact ubiquinol:cytochrome C oxidoreductase (3 subunits) (19) Detection of the F_v fragment via immunoelectron microscopy (53, 54) and immunofluorescence microscopy (56)
V_H domain of the photosystem I-binding 1E7 F_v fragment (mouse hybridoma)	Purification of the intact F_v fragment (55) Capture of the intact photosystem I (3 subunits) (55) Detection of the F_v fragment via Western blotting (55) and immunoelectron microscopy (55)
Phytochrome A (oat)	Purification of the functional apo-protein (expressed in yeast) (17)
Alkaline phosphatase *(E. coli)*	Purification of the active metalloenzyme (32)
Green fluorescent protein *(A. victoria)*	Purification of the functional protein (2) Detection in Western blot
Subunit B of *H. pylori* urease	Copurification of expressed A and B subunits (4) Detection in Western blot (Subunit B)
Curli assembly factor CsgG (*E. coli* outer membrane)	Purification of the native outer membrane protein (8)
Phytochrome Cph1 (Cyanobacteria)	Purification of the active, light-regulated histidine kinase
Glutathione S-transferase and fusion proteins	Purification of the dimeric fusion enzyme (33) Detection in ELISA (33)
V_H domain of the cytochrome C oxidase-binding 7E2C50S F_v fragment (mouse hybridoma)	Purification of the intact F_v fragment (38) Capture of the cytochrome c oxidase subunits I and II (38)
Hydrogenase precursor *(E. coli)*	Purification of the active precursor with N-terminal *Strep*-tag II (25)
PdxA and PdxJ *(E. coli)*	Purification of the active enzymes for vitamin B6 synthesis (23)
Hyaluronate synthase (group A streptococci)	Purification of the active enzyme, probably in membrane-anchored form (1)
SP1 and SP3 transcription factors	Purification of the soluble proteins (expressed in SF9 cells) (1)
Ligase I, III, IV, and XRCC4 (human)	Purification of the active enzymes (expressed in HeLa cells) (1)
T7 RNA polymerase *(E. coli)*	Purification of the active enzyme (1)
CPP32 (human)	Purification of the active cysteine protease (1)
BetP *(C. glutamicum)*	Purification of this integral membrane protein for glycine betaine uptake exhibiting the same transport characteristics compared to the wild-type protein (39)
NADH dehydrogenase fragment of the so called complex I *(E. coli)*	Purification of the correctly assembled complex composed of 3 subunits, one containing the *Strep*-tag II (40)
BHLHzip domain of c-Myc (human)	Purification of the correctly folded protein domain binding to its cognate ligand "Max" (21)
Tissue transglutaminase, the major autoantigen of gluten-sensitive enteropathy (human)	Purification of the active enzyme which had been expressed in the human embryonic kidney cell line 293-EBNA (14)

Large proteins

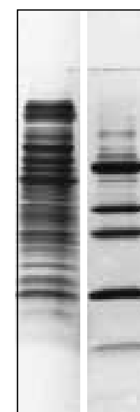
Oat phytochrome A, 124 kDa



Reference:
Murphy J T & Lagarias J C, 1997: Photochem. Photobiol., 65, 750-758. Purification and characterization of recombinant affinity peptide-tagged oat phytochrome A.

Multimeric membrane protein complexes

P. denitrificans cytochrome c oxidase



Reference:
Kleymann G, Ostermeier C, Ludwig B, Skerra A & Michel H, 1995: Bio/ Technology 13, 155-160. Engineered Fv fragments as a tool for the one-step purification of integral multisubunit membrane protein complexes.

Metalloenzymes

E. coli alkaline phosphatase

Reference:
Hengsakul M, Cass AEG, 1997: J. Mol. Biol. 266: 621-632.
Alkaline phosphatase-*Strep*-tag fusion protein binding to streptavidin: Resonant mirror studies.

References:
Skerra A, Schmidt TGM, 1999: Biomolecular Engineering 16: 79-86. Applications of a Peptide Ligand for streptavidin: the Strep-tag.
Skerra A, Schmidt TGM, 2000: Meth. Enzymol. 326: 271-304. Use of the Strep-tag and streptavidin for recombinant protein purification and detection.

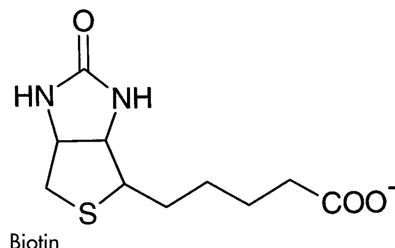
Strep-tag® purification system

Pure and functional proteins after one simple chromatographical step

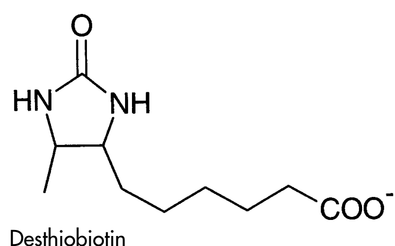
Features and benefits

- Simple one-step purification from crude lysate to > 99% pure protein under physiological conditions possible (see figure on page 22)
- High and selective binding activity and high capacity due to special *Strep*-Tactin® resins
- Resins for gravity flow, low pressure or HPLC applications
- Co-purification of non-covalently bound ligands, thus, protein-protein interaction studies are possible (see page 18)
- Column regeneration and activity status is visualized by color change

Application	Recommended resins	Page
gravity flow	*Strep*-Tactin Sepharose®	22
gravity flow, low pressure or FPLC	*Strep*-Tactin Superflow®	23
gravity flow, low pressure or FPLC	*Strep*-Tactin Macroprep®	24
FPLC or HPLC	*Strep*-Tactin POROS® 20/50	25



Biotin



Desthiobiotin

Principle and properties

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 bioactivity. The *Strep*-tag system can be used to purify *Strep*-tag II proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria (see references page 35).

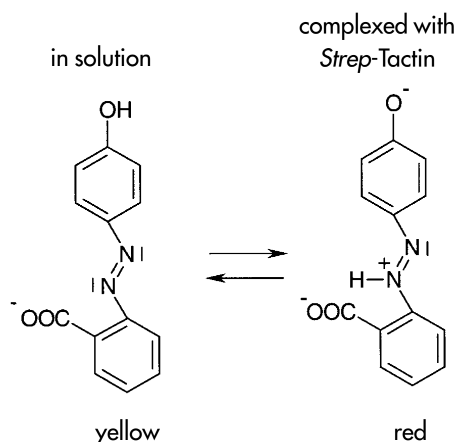
Vectors for bacterial expression are described on pages 41 ff.

Procedure

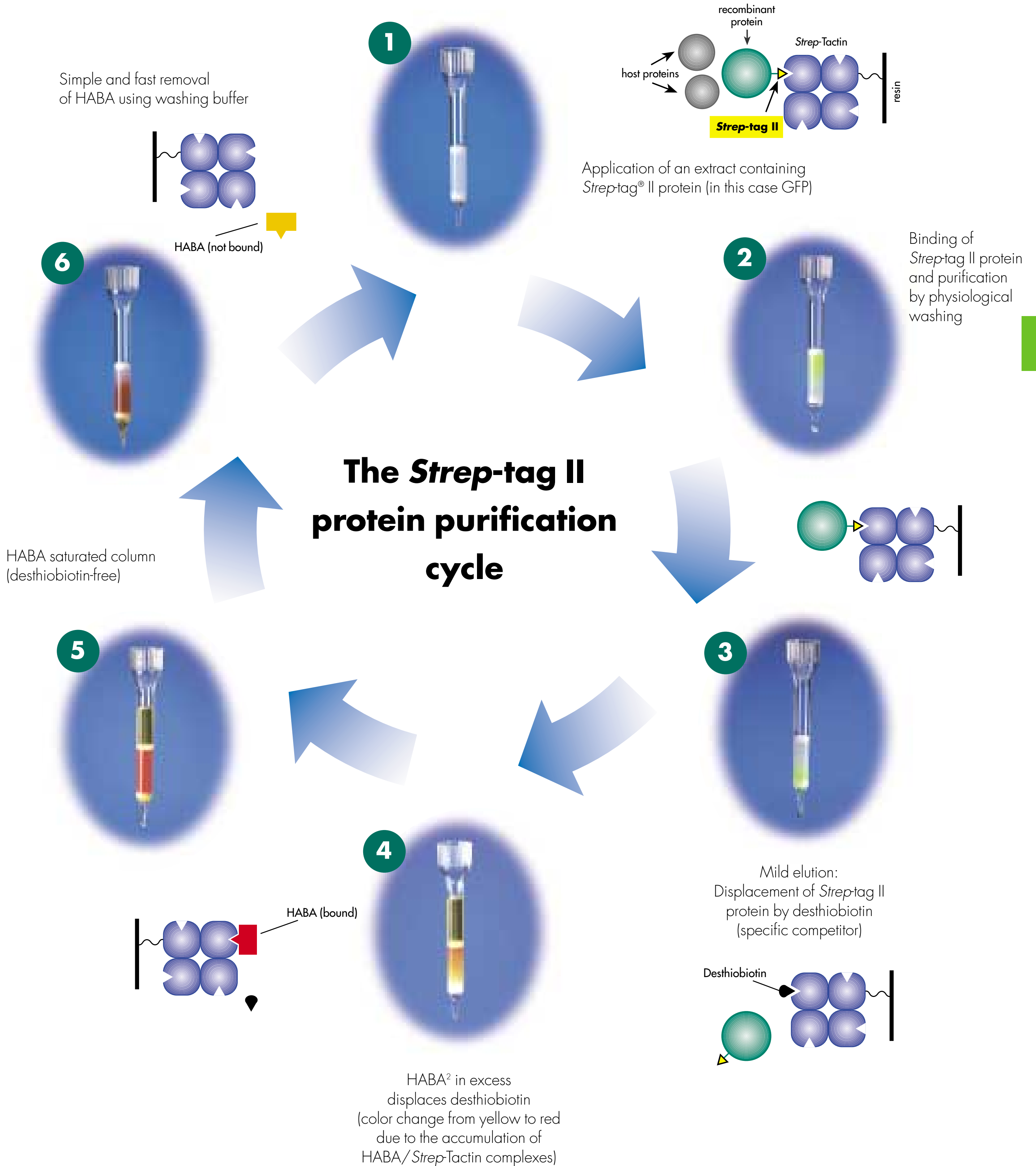
The purification of *Strep*-tag II fusion proteins is very simple and user-friendly. The complete procedure can be performed under nearly physiological conditions, e.g. in PBS buffer and PBS/2.5 mM desthiobiotin buffer during elution. On page 17 the procedure is documented for the purification of GFP (Green Fluorescent Protein).

- Once the tagged protein has bound specifically to the column the unspecific proteins are rapidly washed away with small amounts of physiological washing buffer (step 2).
- Then, small amounts of the specific competitor desthiobiotin are added in order to elute the *Strep*-tag protein (step 3). Since the buffer conditions during elution essentially remain unchanged, potentially unspecifically binding proteins (without *Strep*-tag) will not be eluted and, thus, will not contaminate the protein of interest.
- To regenerate the column, desthiobiotin is displaced by adding the yellow solution HABA (2-[4'-hydroxy-benzeneazo] benzoic acid). After the removal of desthiobiotin the column turns red due to the binding of HABA as red colored hydrazone isomer to *Strep*-Tactin. This clear color change indicates the regeneration and activity status of the column (steps 4 + 5).
- HABA can be removed simply by using washing buffer. Once the red color has disappeared the column can be re-used (steps 6 + 1).

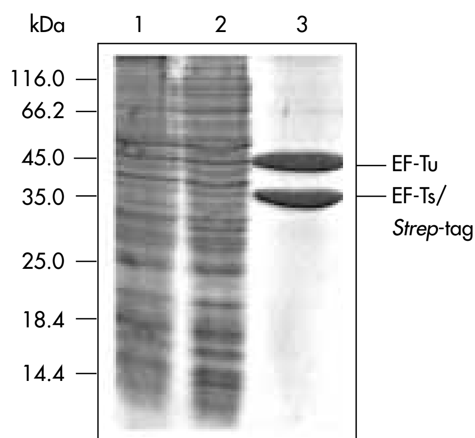
For desthiobiotin and buffer order information see page 28.



HABA
(2-[4'-hydroxy-benzeneazo] benzoic acid)



[1] Please note, that the resin used in this example is *Strep*-Tactin Sepharose. Other resins, such as Strep-Tactin MacroPrep, may not show such a clear color change (see page 20).
[2] 2-[4'-hydroxy-benzeneazo] benzoic acid



The recombinant *Strep*-tag protein is expressed in *E. coli* and binds to a specific partner. The whole complex is purified via *Strep*-tag.

The elongation factor Ts (EF-Ts) fused to *Strep*-tag has been expressed in *E. coli*. Lane 1 shows the protein content of total cells after expression. The soluble part (lane 2) is subjected to *Strep*-Tactin affinity chromatography. Elution (lane 3) reveals that EF-Ts is isolated complexed with EF-Tu being the authentic binding partner of EF-Ts.

Applications

The *Strep*-tag® protein purification system provides the reliable one-step purification of proteins suitable for any application, including:

- Structural and functional investigations
- Crystallization for determination of 3D structure
- Immunization to produce antibodies

Due to the mild conditions *Strep*-tag II recombinant proteins can also be used for:

- Assays involving protein-protein and protein-DNA interactions
- Investigating ligand-receptor interactions under physiological conditions
- Separating living cells for re-culturing purposes

Protein-Protein Interactions

Strep-tag as a tool for studying protein-protein interactions

The isolation of unknown binding partners of a protein which is known to be part of a certain metabolic pathway is a key issue e.g. in drug discovery. Due to its mild purification procedure *Strep*-tag is ideally suited for the isolation of protein complexes and, therefore, for such attempts as described above. This has been exemplarily shown for the elongation Factor - Ts, where the recombinant *Strep*-tag fusion protein expressed in *E. coli* formed a complex with natural *E. coli* proteins. The intact complex could be purified by *Strep*-Tactin® affinity chromatography as the correct stochiometry of the binding partners was found in the eluate.

For a combination of *Strep*-tag with 6xHis-tag in double tag proteins please refer to page 37 ff.

Strep-tag® Starter Kits

Get started with *Strep*-tag

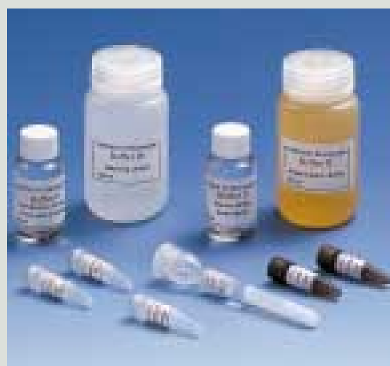
Attractive offers for newcomers are our *Strep*-tag Starter Kits containing all essential reagents required for expression in *E. coli*, purification and detection of *Strep*-tag proteins. While the regular Starter Kit contains one *Strep*-Tactin purification column, the Starter Kit 3C includes 3 different columns with *Strep*-Tactin® immobilized to Sepharose®, MacroPrep® and Superflow®, respectively, allowing the evaluation of the optimal resin for your particular protein of interest. See also *Strep*-Tactin Column Evaluation Set on page 26.

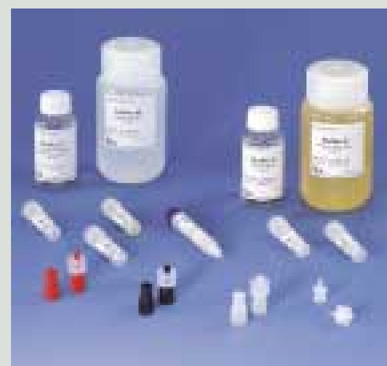
The new *Strep*-tag Starter Kit "Cartridge" contains one of our new *Strep*-Tactin MacroPrep or Superflow 1 ml cartridges for protein purification under low pressure (see also pages 21, 23, 24) allowing fast and convenient purification of your *Strep*-tag protein. The column is delivered pre-packed and is compatible with syringes, peristaltic pump chromatography systems or FPLC/HPLC workstations. Four different sets of the required adapters are included in the kit (m & f, see also page 21) allowing a direct connection to the most common systems.

The kits include the complete set of reagents essential for the expression, purification and detection of *Strep*-tag proteins. For the appropriate expression vector of choice we offer 50% discount if purchased in combination with a Starter Kit. An overview of the vectors presently available is presented on page 44. For applications and specifications of the columns as well as their regeneration, see pages 16, 18, 20.

The *Strep*-Well HT Purification Starter Kit is described on page 53.
The *Strep*/His Starter Kit is described on page 40.

50 % discount on one vector* of choice with the purchase of a Starter Kit!





New

Strep-tag Starter Kit 1 purification column	*Strep*-tag Starter Kit 3C 3 different purification columns	*Strep*-tag Starter Kit "Cartridge" 1 cartridge for purification under low pressure
Kit contents	Kit contents	Kit contents
1 gravity flow column with *Strep*-Tactin Sepharose, 1 ml	3 gravity flow columns with *Strep*-Tactin Sepharose, *Strep*-Tactin MacroPrep and *Strep*-Tactin Superflow, respectively (1ml each)	1 *Strep*-Tactin MacroPrep or Superflow cartridge, 1 ml; optionally 4 adapter sets for connection to peristaltic pump chromatography systems or FPLC/HPLC workstations

Included in all kits (sufficient for 8 applications):
- control plasmid with 15 kD protein insert
- anhydrotetracycline for induction of expression
- fractionation buffer for the preparation of a periplasmic extract
- washing buffer for column chromatography or for the preparation of a cytoplasmic extract
- elution buffer for displacing the *Strep*-tag protein from the column
- column regeneration buffer (with HABA)
- *Strep*-Tactin horse radish peroxidase (HRP) conjugate for Western blot detection
- Comprehensive manual

cat. no.	cat. no.	cat. no.
2-1101-000	2-1102-000	2-1103-000 MacroPrep cartridge kit with adapters 2-1104-000 MacroPrep cartridge kit without adapters 2-1105-000 Superflow cartridge kit with adapters 2-1106-000 Superflow cartridge kit without adapters

* vectors are described on pages 41 ff.

Strep-Tactin® resin specifications

Strep-Tactin Column Evaluation Set: *Strep*-Tactin MacroPrep, Superflow and Sepharose (left to right). Scale: reduced. Order information see page 26.

Strep-Tactin columns regenerated with HABA (see also pages 12, 17): *Strep*-Tactin MacroPrep, Superflow and Sepharose (left to right). Since MacroPrep is not as transparent as Sepharose or Superflow, the color shift to red is less visible. Scale: reduced.

Five *Strep*-Tactin resin versions are available which differ in their properties and applications. While *Strep*-Tactin Sepharose® is preferentially used for gravity flow chromatography, *Strep*-Tactin Superflow® and *Strep*-Tactin MacroPrep® can also be used for low pressure or FPLC applications, and *Strep*-Tactin POROS® (20 and 50) for FPLC and HPLC chromatography. Each matrix exhibits different non-specific binding properties. In cases where unsatisfactory results are obtained with one matrix due to unspecific background, it is advisable to switch to one of the other matrices (see also *Strep*-Tactin Column Evaluation Sets, page 26. In addition, Superflow is especially suited for increased flow rates and is also useful for the purification of large protein complexes (see page 23).

Please note, that the *Strep*-Tactin resins are optimized for column affinity chromatography as opposed to batch purification. Our pre-packed columns are described on the following pages. For batch purification use our Mag*Strep* *Strep*-Tactin coated Magnetic Beads (as described on page 27).

In table 1 (below), reagents compatible with the *Strep*-tag/*Strep*-Tactin interaction are listed.

For order information and further details on the columns and matrices please refer to the following pages.

Table 1: Reagents compatible with the *Strep*-tag/*Strep*-Tactin interaction

Reagent	Concentration
β-mercaptoethanol	5 mM
CHAPS	0.1 %
$C_{10}E_6$	0.03 %
$C_{12}E_8$	0.005 %
DTT	5 mM
EDTA	10 mM
Guanidine	0.5 M
Glycerol	10 %
Imidazole	250 mM
NaCl	1 M
N -dodecyl β-D-maltoside (LM)	0.007 %
N -dodecyl-N,N-dimethylamine-N-oxide (LDAO)	0.7 %
N -lauryl sarcosine	0.1 %
N -nonyl β-D-glucopyranoside (NG)	0.2 %
N -octyl β-D-glucopyranoside (OG)	0.005 %
Sodium-N -dodecyl sulfat (SDS)	0.01 %
Triton X-100 (TX)	0.1 %
Tween 20	0.1 %
Urea	0.5 M

Table 2: Specifications of *Strep*-Tactin resins

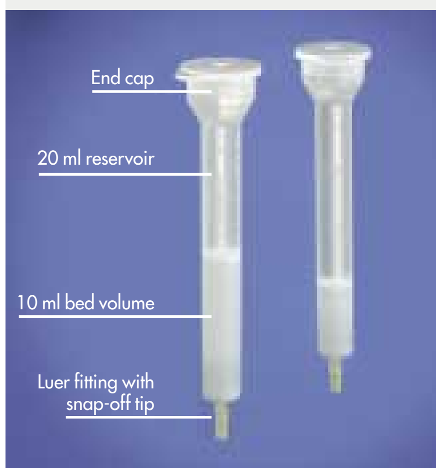
	Strep-Tactin Sepharose	*Strep*-Tactin Superflow	*Strep*-Tactin MacroPrep	*Strep*-Tactin POROS 20	*Strep*-Tactin POROS 50
Gravity flow column	Yes	Yes	Yes	No	No
FPLC	No	Yes	Yes	Yes	Yes
HPLC	No	Yes	Yes	Yes	Yes
Binding capacity	50 - 100 nmol/ml	50 - 100 nmol/ml	50 - 100 nmol/ml	25 - 50 nmol/ml	25 - 50 nmol/ml
Support	Sepharose 4FF	Superflow 6	MacroPrep 50	POROS 20	POROS 50
Bead structure	agarose	6% agarose, crosslinked	polymethacrylate	polystyrene	polystyrene
Bead size	45 - 165 µm	60 - 160 µm spherical	50 µm	20 µm	50 µm
Exclusion limit	3×10^7	6×10^6	1×10^6	n.d.	n.d.
Recommended linear flow rate[1]	up to 30 cm/h	up to 300 cm/h	up to 300 cm/h	300 - 500 cm/h	300 - 500 cm/h
pH stability	4 - 9	2 - 13	2 - 12	1 - 13	1 - 13
	Extreme pH as listed above may be used for short cleaning procedures but should be changed to pH 8.0 for storage and *Strep*-tag protein binding				
Max. pressure	Gravity flow	n.d	n.d	170 bar	170 bar
Form	buffered 50% suspension	buffered 50% suspension	buffered 50% suspension	buffered 50% suspension	buffered 50% suspension
Storage	4 °C, do not freeze	4 °C, do not freeze	4 °C, do not freeze	4 °C, do not freeze	4 °C, do not freeze
Shipment	RT	RT	RT	RT	RT

n.d. not determined; [1]linear flow rate calculation see page 123.

Strep-Tactin® column formats

Gravity flow column with 0.2 ml *Strep*-Tactin resin (see pages 22-24). Scale: reduced.

Gravity flow column with 1 ml *Strep*-Tactin resin (see pages 22-24). Also available with Ni-NTA resin (see pages 39, 40) Scale: reduced.



5 ml and 10 ml gravity flow columns with *Strep*-Tactin resin (see pages 22-24). 5 ml column also available with Ni-NTA resin (see pages 39, 40). Scale: reduced.

Pre-packed column with *Strep*-Tactin POROS® and accessories (see page 25). Scale: reduced.

Cartridges with 1 ml or 5 ml *Strep*-Tactin Superflow® or *Strep*-Tactin MacroPrep® for low pressure chromatography (see pages 23, 24). Cartridges can be connected in series to enlarge capacity, and can be used with syringes. Adapters are available for use with peristaltic pump chromatography systems or FPLC/HPLC workstations (see table on the right). The cartridges have a female luer lock inlet and a male luer lock outlet. Also available with Ni-NTA resin (see pages 39, 40). Scale: reduced.

Strep-Well HT Purification Plate with immobilized *Strep*-Tactin for high-throughput protein purification (see page 51ff.). Scale: reduced.

Handling and regeneration of *Strep*-Tactin gravity flow columns

Gravity flow columns cannot run dry allowing a step-by-step purification procedure without the need for further equipment. After purification, columns are regenerated with a yellow regeneration buffer containing 1 mM HABA (2-[4'-hydroxy-benzeneazo] benzoic acid; see "buffers and reagents" on page 28). The regeneration process on the column can be followed visually by color change from yellow to red. Prior to the next run the dye is washed off with fast kinetics by equilibration buffer. Please note, that MacroPrep and POROS* do not turn as red as Sepharose (see illustration on page 20) and may keep a slight pink color after regeneration. The binding, elution and regeneration process is described in detail on pages 16 and 17. For details please refer to the *Strep*-tag manual (for download at www.iba-go.com/download.html).

*Color change not visible on non-transparent FPLC/HPLC columns (on the left and on page 25).

Comparison of the different *Strep*-Tactin column formats

Column volume	Protein extract volume
0.2 ml	0.1-2 ml
1 ml	0.5-10 ml
5 ml	2.5-50 ml
10 ml	5-100 ml

Adjust protein extract volume according to binding capacity of the column (see specifications on pages 20, 22-25.

Note: The columns illustrated here are pre-packed with *Strep*-Tactin Sepharose. They are also available with other resins, such as *Strep*-Tactin Superflow (see page 23), *Strep*-Tactin MacroPrep (see page 24) or Ni-NTA resins (see pages 39, 40). Please note, that each resin looks slightly different.

Adapters for cartridges

M6 adapter set for Amersham Biosciences FPLC other than Äkta
Contains: 3x "Luer male to M6 female" and 3x "Luer female to M6 female"

1/4-28 adapter set for FPLC other than Amersham Biosciences
Contains: 3x "Luer male to 1/4-28 female" and 3x "Luer female to 1/4-28 female"

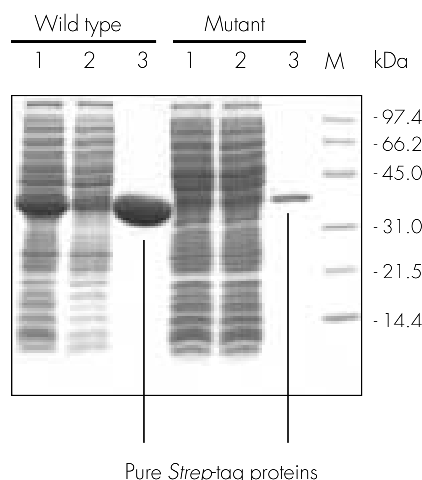
10-32 adapter set for HPLC and Amersham Biosciences Äkta
Contains: 3x "Luer male to 10-32 female" and 3x "Luer female to 10-32 female"

1/16 inch adapter set for peristaltic pump tubing
Contains: 20x "Luer male to barb 1/16 inch" and 20x "Luer female to barb 1/16 inch"

Order info

adapter sets	amount	cat. no.
M6 adapter set for Amersham Biosciences FPLC other than Äkta	1 kit	2-1012-000
1/4-28 adapter set for FPLC other than Amersham Biosciences	1 kit	2-1013-000
10-32 adapter set for HPLC and Amersham Biosciences Äkta	1 kit	2-1014-000
1/16 inch adapter set for peristaltic pump tubing	1 kit	2-1015-000

In one step: over 99% purity and high purification factors



Pure *Strep*-tag proteins

A 36 kDa enzyme and a mutant, both with C-terminal *Strep*-tag II, were expressed in the cytoplasm of *E. coli*. The crude lysate was chromatographically separated on *Strep*-Tactin Sepharose (5 mg/ml) under gravity flow and physiological conditions (100 mM Tris-Cl, pH 8.0). The purification is documented on a Coomassie stained SDS gel where samples from the crude lysate (lane 1), from the flow through (lane 2), and from the elution with 2.5 mM desthiobiotin (lane 3) had been applied. The wild type enzyme is shown to be over 99 % pure (lane 3 contains 50 µg protein vs 0.5 µg protein per band in the molecular size standard). The mutant - although expressed at low level only - was obtained at high purity under the same conditions.

1: Sample of crude lysate after cytosolic expression with pASK-IBA3
2: Sample of flow through during chromatography
3: Sample after adding 2.5 mM desthiobiotin
M: Molecular size standard (kDa)

Further specifications of column formats see page 21.

Strep-Tactin® Sepharose®

for purification of *Strep*-tag® II proteins by gravity-flow column chromatography

Strep-Tactin Sepharose provides high binding capacity and minimal non-specific binding (see figure on the left). The material can be used for gravity flow purification.

Since the *Strep*-Tactin resins are optimized for column affinity chromatography as opposed to batch purification, we do recommend our pre-packed *Strep*-Tactin Sepharose columns, which are available in different formats (from 0.2 ml mini columns up to 10 ml columns). Further specifications on the various column formats can be found on page 21. For packing individual columns bulk material is available.

Specifications of *Strep*-Tactin Sepharose resin

Strep-Tactin concentration	5 mg/ml sedimented resin
Binding capacity	1 ml sedimented resin is useful for one-step purification of 50 to 100 nmol recombinant protein (up to 3 mg in case of a 30 kDa protein)
Support	Sepharose 4FF, 4 % agarose
Bead size	45 - 165 µm
Linear flow rate[1]	gravity flow
Exclusion limit	3 x 10^7 Da
Form (bulk)	50 % suspension in 100 mM Tris/HCl pH 8.0, 1 mM EDTA, 150 mM NaCl
Stability	at least 6 months after shipment
Storage	4 °C, DO NOT FREEZE
Shipment	RT

For detailed information on the *Strep*-tag purification system see page 16 ff.

product	amount	cat. no.
Gravity flow *Strep*-Tactin Sepharose mini-columns (0.2 ml bed volume)	5 columns	2-1202-505
Gravity flow *Strep*-Tactin Sepharose column (1 ml bed volume)	1 column	2-1202-001
	5 columns	2-1202-005
Gravity flow *Strep*-Tactin Sepharose column (5 ml bed volume)	1 column	2-1202-051
Gravity flow *Strep*-Tactin Sepharose column (10 ml bed volume)	1 column	2-1202-101
Strep-Tactin Sepharose (50 % suspension)	20 ml	2-1201-010
	50 ml	2-1201-025
	200 ml	2-1201-100
	1000 ml	2-1201-500

Strep-tag protein purification buffer set see page 28

[1] calculation see page 123

Strep-Tactin® Superflow®

New

4.8 MDa VLPs purified!

for purification of *Strep*-tag® II proteins by gravity flow, low pressure and FPLC

Strep-Tactin Superflow combines superior mechanical stability and outstanding flow characteristics with high dynamic binding capacity. It can be used for gravity flow as well as for FPLC applications, is suitable for increased flow rates and is also useful for the purification of large protein complexes (see special application below). Since the *Strep*-Tactin resins are optimized for column affinity chromatography as opposed to batch purification, we do recommend our pre-packed *Strep*-Tactin Superflow columns. They are available in different formats (from 0.2 ml mini columns up to 10 ml columns) and are designed for gravity flow. For more convenient use, *Strep*-Tactin Superflow is also available pre-packed in cartridges. Please refer to pages 21 and 24 for more information on the column and cartridge formats. For packing individual columns bulk material is available.

Specifications of *Strep*-Tactin Superflow resin

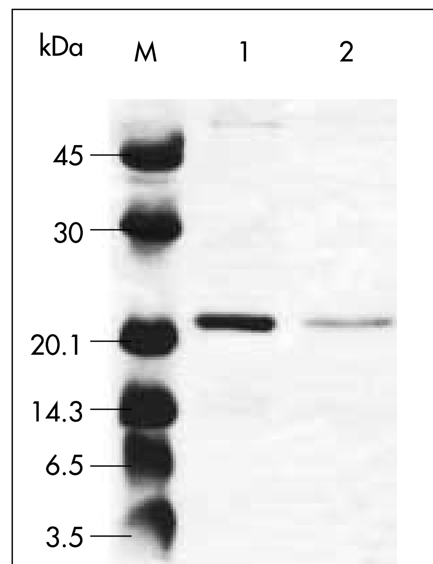
Binding capacity	1 ml sedimented resin (corresponding to 2 ml of a 50 % suspension) can be used for one-step purification of 50 to100 nmol recombinant protein (up to 3 mg in case of a 30 kDa protein)
Support	Superflow 6 (6 % agarose, crosslinked)
Bead size	60-160 µm, spherical
Linear flow rate[1]	100-300 cm/h is recommended for *Strep*-tag purification
Form (bulk)	50 % suspension in 100 mM Tris/HCl pH 8.0, 150 mM NaCl, 1 mM EDTA
Stability	at least 6 months after shipment
Storage	4°C, DO NOT FREEZE
Shipment	RT

For detailed information on the *Strep*-tag purification system see page 16 ff.

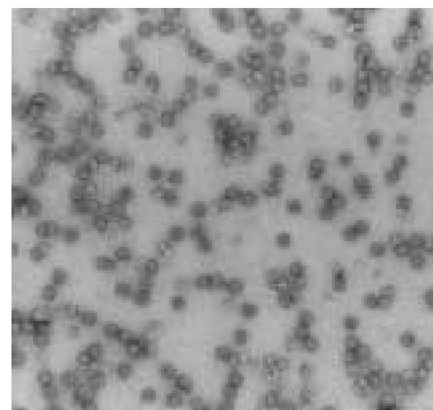
product	amount	cat. no.
Gravity flow *Strep*-Tactin Superflow mini columns (0.2 ml bed volume)	5 columns	2-1207-505
Gravity flow *Strep*-Tactin Superflow column (1 ml bed volume)	1 column	2-1207-001
	5 columns	2-1207-005
Gravity flow *Strep*-Tactin Superflow column (5 ml bed volume)	1 column	2-1207-051
Gravity flow *Strep*-Tactin Superflow column (10 ml bed volume)	1 column	2-1207-101
***Strep*-Tactin Superflow cartridge** (1 ml bed volume)	1 cartridge	2-1211-001
***Strep*-Tactin Superflow cartridge** (1 ml bed volume)	5 cartridges	2-1211-005
***Strep*-Tactin Superflow cartridge** (5 ml bed volume)	1 cartridge	2-1212-001
***Strep*-Tactin Superflow cartridge** (5 ml bed volume)	5 cartridges	2-1212-005
***Strep*-Tactin Superflow** (50 % suspension)	20 ml	2-1206-010
	50 ml	2-1206-025
	200 ml	2-1206-100
	1000 ml	2-1206-500

Strep tag protein purification buffer set see page 28

Intact VLP purification using *Strep*-Tactin Superflow



Left to right: M; Rainbow Marker RPN 755, Amersham Pharmacia Biotech, 1 and 2; different elution fractions of VLPs fused to *Strep*-tag II after one-step *Strep*-Tactin affinity purification from a crude lysate of *E. coli*. Due to the denaturing conditions in the gel the capsides are separated into monomers (20 kDa each).

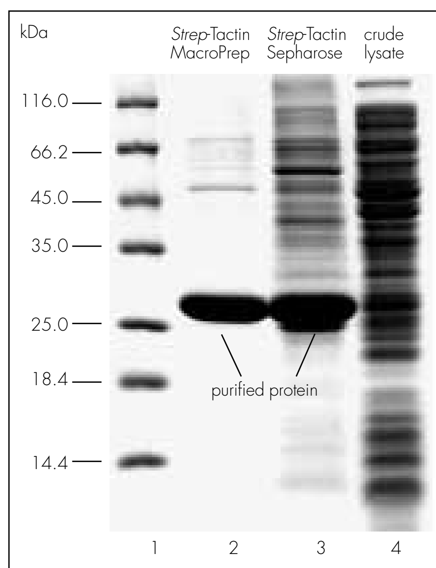


Electron microscopy preparations of intact VLPs fused to *Strep*-tag II after purification on *Strep*-Tactin Superflow, uranyl acetate stain, 40 000x. The 30 nm capsides (4.8 MDa) consist of 240 monomers each.

Illustrations were kindly provided by L. Stöckl and B. Brandenburg, Robert Koch Institute, Berlin. Further details at www.iba-go.com.

[1] calculation see page 123

For some recombinant proteins, purification characteristics on *Strep*-Tactin MacroPrep differ from those on *Strep*-Tactin Sepharose:



The Coomassie stained SDS gel shows the purification of a recombinant protein which exhibits exceptionally high non-specific protein binding. In this case, we have been able to remove the contaminants nearly quantitatively using the *Strep*-Tactin MacroPrep resin while the contaminants co-eluted with the recombinant protein after the same purification protocol using the *Strep*-Tactin Sepharose resin. (Please note, that normally behaving recombinant proteins can be purified in a single step to homogeneity using *Strep*-Tactin Sepharose; see page 22). This example shows that it may be reasonable to change the resin if non-specific protein binding occurs.
Lane 1, molecular weight standard (kDa);
lane 2, *Strep*-Tactin MacroPrep purified protein sample;
lane 3, *Strep*-Tactin Sepharose purified protein sample;
lane 4, soluble raw lysate as applied on each column.

New

***Strep*-Tactin MacroPrep cartridges for rapid purification under low pressure (see also page 21)**

Strep-Tactin® MacroPrep®

for purification of *Strep*-tag® II proteins by gravity flow or under low pressure

Strep-Tactin MacroPrep is suited for all low pressure chromatography applications. The MacroPrep resin exhibits non-specific binding properties differing from those of Sepharose® or Superflow® and can, thus, be recommended in cases where suboptimal results are obtained with Sepharose or Superflow and vice versa (see figure on the left). See also *Strep*-Tactin Column Evaluation Set on page 26.

Since the *Strep*-tag purification system is designed for column affinity chromatography as opposed to batch purification, we do recommend our pre-packed *Strep*-Tactin MacroPrep devices for column chromatography.

Our gravity flow columns are available in different sizes with a bed volume of 0.2 ml, 1 ml, 5 ml and 10 ml and can be used without the need for any further special equipment.

Our *Strep*-Tactin MacroPrep cartridges are pre-packed, ready-to-use chromatography columns for convenient use with syringes, peristaltic pump chromatography systems or FPLC/HPLC workstations for rapid and automated purification of *Strep*-tag fusion proteins under low pressure.

Strep-Tactin MacroPrep cartridges are available in 1 ml and 5 ml formats and can be connected in series to enlarge capacity. Adapters for the luer in- and outlets are available and described on page 21. The average capacity of the 1 ml *Strep*-Tactin MacroPrep cartridge is 50-100 nmol recombinant *Strep*-tag fusion protein per run and the recommended flow rate is 1 ml/minute (equivalent to approximately 20 drops per minute when working with a syringe).

Specifications of *Strep*-Tactin MacroPrep resin

Binding capacity	1 ml sedimented resin (corresponds to 2 ml of a 50 % suspension) can be used for one-step purification of 50 to 100 nmol recombinant protein
Support	MacroPrep® 50 (polymethacrylate)
Bead size	50 µm
Linear flow rate[1]	Up to 300 cm/h can be used for *Strep*-tag purification
Form (bulk)	50 % suspension in 100 mM Tris/HCl pH 8.0, 1mM EDTA, 150 mM NaCl
Stability	at least 6 months after shipment
Storage	4°C, DO NOT FREEZE
Shipment	RT

For detailed information on the *Strep*-tag purification system see page 16 ff.

product	amount	cat. no.
Gravity flow *Strep*-Tactin MacroPrep mini-columns (0.2 ml bed volume)	5 columns	2-1506-505
Gravity flow *Strep*-Tactin MacroPrep column (1 ml bed volume)	1 column	2-1506-001
	5 columns	2-1506-005
Gravity flow *Strep*-Tactin MacroPrep column (5 ml bed volume)	1 column	2-1506-051
Gravity flow *Strep*-Tactin MacroPrep column (10 ml bed volume)	1 column	2-1506-101
***Strep*-Tactin MacroPrep Cartridge** (1 ml bed volume)	1 cartridge	2-1511-001
	5 cartridges	2-1511-005
***Strep*-Tactin MacroPrep Cartridge** (5 ml bed volume)	1 cartridge	2-1512-001
	5 cartridges	2-1512-005
***Strep*-Tactin MacroPrep** (50 % suspension)	20 ml	2-1505-010
	50 ml	2-1505-025
	200 ml	2-1505-100
	1000 ml	2-1505-500

Strep-tag protein purification buffer set see page 28

[1] calculation see page 123

Strep-Tactin® POROS®

for purification of *Strep*-tag® II proteins by FPLC or HPLC

Strep-Tactin POROS optimally combines high flow rates with selective binding and high dynamic capacity (see figures on the right). This material can be used for FPLC or HPLC applications allowing fast *Strep*-tag protein purification.[1] *Strep*-Tactin POROS 20 has a bead size of 20 µm, while POROS 50 has a bead size of 50 µm. The binding capacity is identical.

Since the *Strep*-Tactin resins are optimized for column affinity chromatography as opposed to batch purification, we do recommend our pre-packed 1.7 ml *Strep*-Tactin POROS columns for FPLC and HPLC. For packing individual columns bulk material is available.

Specifications of *Strep*-Tactin POROS resin

Binding capacity	1.7 ml column can be used for the one-step purification of 40 to 80 nmol recombinant protein
Support	POROS 20 or POROS 50
Bead size	20 µm or 50 µm
Linear flow rate[2]	300 - 500 cm/h is possible for *Strep*-tag purification
Form (bulk)	50 % suspension in 100 mM Tris/HCl pH 8.0, 1mM EDTA, 150 mM NaCl
Stability	at least 6 months after shipment
Storage	4°C, DO NOT FREEZE
Shipment	RT

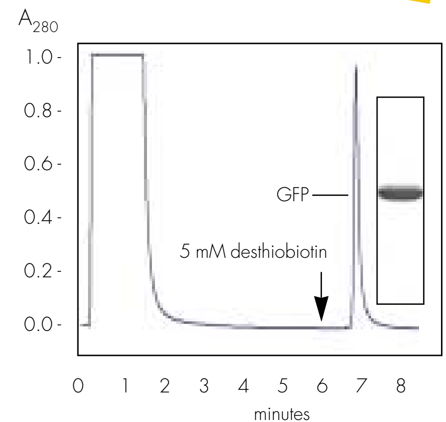
For detailed information on the *Strep*-tag purification system, see page 16 ff.

product	amount	cat. no.
Ready-to-use *Strep*-Tactin POROS 20 column (1.7 ml bed volume)	1 column	2-1203-017
Ready-to-use *Strep*-Tactin POROS 50 column (1.7 ml bed volume)	1 column	2-1205-017
Strep-Tactin POROS 20 (50 % suspension)	2 ml	2-1203-001
	4 ml	2-1203-002
	10 ml	2-1203-005
	20 ml	2-1203-010
Strep-Tactin POROS 50 (50% suspension)	2 ml	2-1205-001
	4 ml	2-1205-002
	10 ml	2-1205-005
	20 ml	2-1205-010

Strep-tag protein purification buffer set see page 28

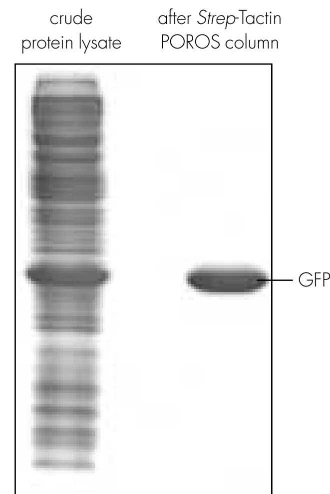
Pre-packed column with *Strep*-Tactin POROS and accessories.

Semi-preparative purification within minutes



The green fluorescent protein (GFP) with C-terminal *Strep*-tag II was expressed in the cytoplasm of *E. coli*. After sterile filtration the crude lysate was separated via Perfusion Chromatography® on *Strep*Tactin POROS (5 mg/ml) under physiological conditions (100 mM Tris-Cl pH 8.0, 1 mM EDTA). Elution was performed by the addition of 5 mM desthiobiotin in the same buffer and chromatography was monitored by measuring the absorbance of the eluate at 280 nm.

crude protein lysate | after *Strep*-Tactin POROS column



The purification is documented on a Coomassie stained SDS gel where samples from the crude lysate (lane 1) and from the elution peak at 6.7 minutes (lane 2) had been applied. 3 mg GFP could be obtained in one step when running a 1.7 ml column on a BioCAD® workstation at 4 ml/minute.

[1] Please note, that the binding capacity is just about half of that of other matrices as described on pages 22-24. POROS is not recommended for membrane proteins.

[2] calculation see page 123

Strep-Tactin Column Evaluation Set: *Strep*-Tactin Sepharose, Superflow and MacroPrep (left to right). Scale: reduced.

3 columns for the price of 2!

Strep-Tactin Mini-Column Evaluation Set. Scale: reduced.

Strep-Tactin® Column Evaluation Sets

Optimize your results!

Each support exhibits different non-specific binding properties. Thus, the appropriate support for the purification of a recombinant protein is normally not predictable and it depends on the recombinant protein, which support works best for purification. For rapid optimization of your purification results, we are now offering two Column Evaluation Sets with *Strep*-Tactin immobilized to the supports Sepharose®, Superflow® or MacroPrep®, respectively. The regular size set contains our standard 1 ml columns, while the Mini-Column Set contains our new 0.2 ml mini-columns for purification of 10-20 nmol protein (illustrations on the left). For further specifications of the matrices please refer to the following pages.

product	amount	cat. no.
Strep-Tactin Mini-Column Evaluation Set 3 *Strep*-Tactin columns with 0.2 ml bed volume each	1 set	2-1001-013
Strep-Tactin Column Evaluation Set 3 *Strep*-Tactin columns with 1 ml bed volume each	1 set	2-1001-003

The *Strep*-Tactin support is either Sepharose, Superflow or MacroPrep.
Shipped at RT; store at 4°C, DO NOT FREEZE.

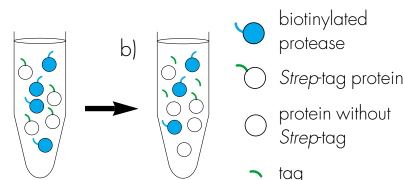
Tips and tricks: *Strep*-tag® removal

Generation of processed protein using biotinylated proteases

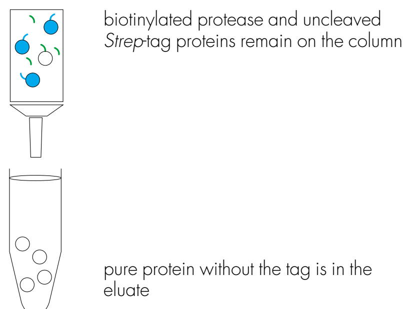
- Incubate your purified recombinant *Strep*-tag protein of interest after removal of desthiobiotin with a commercially available biotinylated endoproteinase*.
- After digestion, apply the mixture on a *Strep*-Tactin column.
- The biotinylated protease as well as potentially uncleaved *Strep*-tag protein will bind to the column.
- Only the cleaved protein without *Strep*-tag will flow through the column.

Convenient method to remove *Strep*-tag from purified proteins

1) Buffer exchange (desthiobiotin removal, optimal protease cleavage conditions)
2) Cleavage with biotinylated protease



3) Purification via a *Strep*-Tactin column



* factor Xa or thrombin

Mag*Strep*: *Strep*-Tactin® coated Magnetic Beads

A convenient tool for batch purification of *Strep*-tag® proteins

Please note that for expression rates below 1 mg/liter culture, i.e. if the target protein concentration in the crude extract is less than 100 µg/ml, we highly recommend to use our *Strep*-Tactin purification columns (see pages 21-26) or for high-throughput applications our *Strep*-Well HT Purification Plates (see pages 51 ff.) instead of performing batch purification with Mag*Strep*.

Mag*Strep* (*Strep*-Tactin coated Magnetic Beads) is a new tool for the fast purification of *Strep*-tag proteins in batch format offering the possibility to work with small amounts of recombinant protein in solution. Within minutes, the target molecules are bound specifically by *Strep*-Tactin. Using a magnetic separator, the Magnetic Beads are separated within seconds from solution.

The Mag*Strep* Kit includes *Strep*-Tactin coated Magnetic Beads as well as washing, incubation and elution buffers optimized for batch purification of *Strep*-tag proteins. Mag*Strep* Beads are available separately as well. For convenience, we are also offering a Magnetic Separator for 24 reaction tubes (12 x 1.5 ml and 12 x 2 ml).

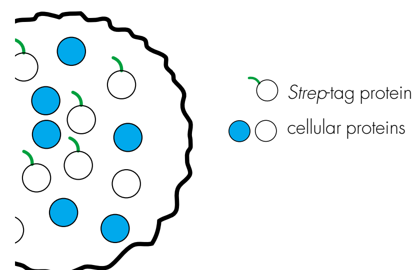
Specifications of Magnetic Beads

Bead size	polydisperse, 0.5 – 1.1 µm
Magnetite	50 – 60 %
Support	M-PVA-beads
Form	50 mg/ml suspension in Dulbecco's PBS; 0.1 % bovine serum albumin; 0.02 % NaN_3
Binding capacity	*Strep*-tag II fusion proteins: approx. 90 – 110 pmol/mg beads
Storage	4°C, DO NOT FREEZE
Shipment	RT

product	amount	cat. no.
Mag*Strep* Kit (incl. 2 ml *Strep*-Tactin coated Magnetic Beads and buffers)	1 kit	2-1601-000
Mag*Strep* Beads (*Strep*-Tactin coated Magnetic Beads, 5 % suspension)	2 ml 5 ml	2-1601-002 2-1601-005
Magnetic Separator for 24 rxn tubes (12 x 1.5 ml and 12 x 2 ml)	1	2-1602-000

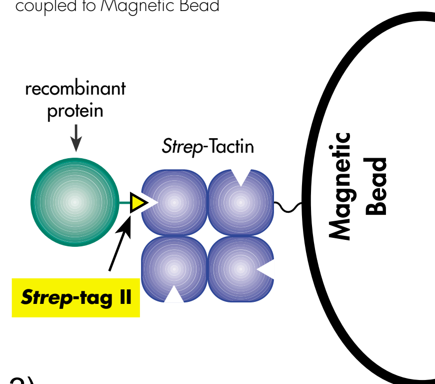
1)

Over-expressed *Strep*-tag fusion protein in an *E.coli* cell extract



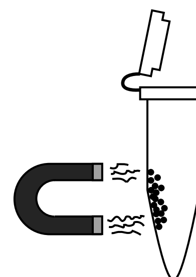
2)

Strep-tag binds *Strep*-Tactin coupled to Magnetic Bead



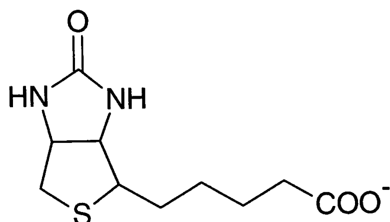
3)

Capture of Magnetic Beads in IBA's magnetic separator

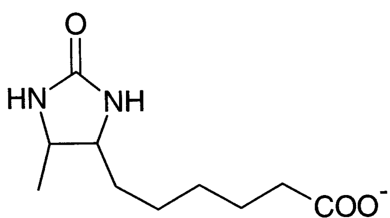


Magnetic Separator

Biotin-free desthiobiotin: thoroughly tested for Strep-tag applications!



Biotin



Desthiobiotin

Buffers and reagents for *Strep*-tag®

Reagents used in combination with the *Strep*-tag technology

Desthiobiotin: Reagent for *Strep*-tag II Elution

Desthiobiotin displaces *Strep*-tag II proteins at the biotin-binding site of *Strep*-Tactin® in a competitive manner resulting in a mild elution of the protein.
For your convenience, we offer desthiobiotin ready-to-use in solution. If you desire to prepare your own elution buffer suitable for other protocols desthiobiotin is offered as lyophilized powder as well.

product	amount	cat. no.
D-Desthiobiotin solution = 10 x Buffer E*	25 ml 10 x buffer for 250 ml	2-1000-025
D-Desthiobiotin	0.107 g for 200 ml 2.5 mM buffer	2-1000-001
	1g	2-1000-002
	5g	2-1000-005

*contains EDTA

Strep-Tactin® Regeneration Buffer with HABA

Contains HABA (2-[4'-hydroxy-benzeneazo] benzoic acid) which displaces desthiobiotin at the biotin-binding site regenerating *Strep*-Tactin. The yellow HABA turns the column red when desthiobiotin has been removed from *Strep*-Tactin clearly indicating the regeneration process and activity status of the column (see pages 12,17).

product	amount	cat. no.
Strep-Tactin regeneration buffer with HABA (10 x concentrated)	100 ml 10 x Buffer R	2-1002-100

Strep-tag Protein Purification Buffer Set

Convenient set for the purification of recombinant *Strep*-tag fusion proteins on immobilized *Strep*-Tactin and for the regeneration of the columns.

product	amount	cat. no.
Strep-tag protein purification buffer set (10 x concentrated buffers)	100 ml 10x Buffer W (W=washing); 25 ml 10x Buffer E (E=elution)*; 100 ml 10x Buffer R (R=regeneration)	2-1002-000

*contains EDTA

New

Biotin Blocking Buffer

For blocking biotinylated proteins in Western blots, the membrane is incubated with Biotin Blocking Buffer. Use a dilution of 1:1000 in standard Western blot blocking reagent prior to detecting *Strep*-tag proteins with *Strep*-Tactin conjugates (see pages 29-32).

product	amount	cat. no.
Biotin Blocking Buffer	2 ml	2-0501-002

Strep-tag® detection system

Highly selective, fast and sensitive

Features and benefits

- Versatile detection of N-terminal, C-terminal or internal *Strep*-tag II
- Selective detection via labeled *Strep*-Tactin® or antibodies
- Reagents suitable for use in Western blot, ELISA, electron microscopy and fluorescence microscopy
- *Strep*-Tactin conjugates for fast protocols (see pages 30, 31)
- *Strep*-tag II specific monoclonal antibody for high specificity (see page 32)

Application	*Strep*-Tactin HRP	*Strep*-Tactin AP	Monoclonal antibody
Dot blot	+ (> 7 ng/dot)	+ (> 1 ng/dot)	+ (> 5 ng/dot)
Western blot	+ (> 7 ng/band)	+ (> 2 ng/band)	+ (> 5 ng/band)
ELISA	+	+	+
Electron microscopy	-	-	+
Fluorescence microscopy	-	-	+

Where determined, sensitivity data are denoted in parantheses. HRP, AP, denote horse radish peroxidase, alkaline phosphatase, respectively.

Principle and properties

The *Strep*-tag detection system is based on labeled *Strep*-Tactin which can be detected directly, or on non-labeled antibodies which must be detected by a secondary antibody that carries the label. Fast detection is possible with *Strep*-Tactin conjugates whereas monoclonal antibody-based detection enables higher specificity. For your convenience IBA offers *Strep*-Tactin detection kits which include all buffers and reagents (see pages 30, 31).

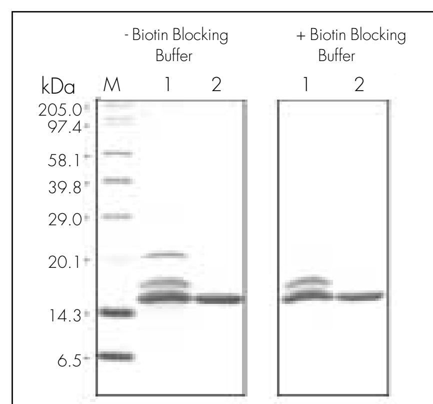
Procedure

Current procedures for equivalent systems based on streptavidin or antibodies can be used. A specific advantage over streptavidin/biotin systems is that the *Strep*-tag is not recognized by avidin. This enables specific blocking of all biotinylated proteins in the sample by IBA's Biotin Blocking Buffer containing avidin (see page 28) and detection of the *Strep*-tag fusion protein only (see Western blot on the right). Our kits include optimized buffers, reagents and protocols for *Strep*-tag detection on Western blots.

Applications

The *Strep*-tag protein detection system provides a means for a broad variety of assays, including:

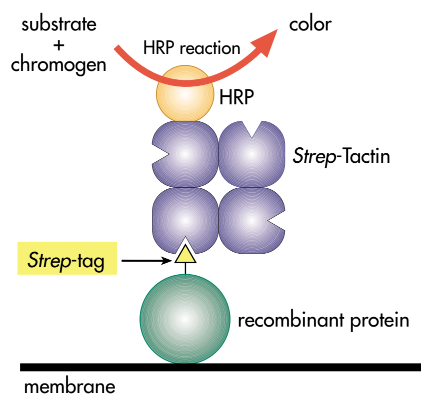
- Colony blot, dot blot, Western blot and ELISA procedures
- Screening for positive expression clones
- Monitoring expression levels and stability of *Strep*-tag proteins
- Immunocytochemistry and immunohistochemistry
- Protein localization and targeting studies

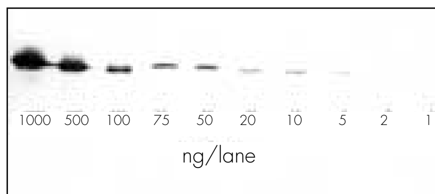


Western blot detection of the azurin/*Strep*-tag II fusion protein with *Strep*-Tactin HRP conjugate. Azurin with a C-terminal *Strep*-tag II was produced at 30 °C using the plasmid pASK-IBA2, and the cytosolic *E. coli* extract was prepared via sonication. 4 µl of this extract (lane 1), 2 µg purified azurin/*Strep*-tag II (lane 2) and a biotinylated molecular size standard (Sigma, lane M; kDa) were separated by 15 % SDS-PAGE. Proteins were transferred to nitrocellulose and, after blocking overnight, the membranes were incubated with *Strep*-Tactin/ HRP conjugate in the presence or absence of Biotin Blocking Buffer. The addition of IBA's Biotin Blocking Buffer (see page 28) almost eliminated the signal resulting from the *E. coli* biotin carboxyl carrier protein (BCCP) at 22.5 kDa. The signals resulting from *Strep*-tag II displayed by the mature azurin (15 kDa) or some of its pre-protein (17 kDa), with the OmpA signal sequence still attached, remained unchanged.

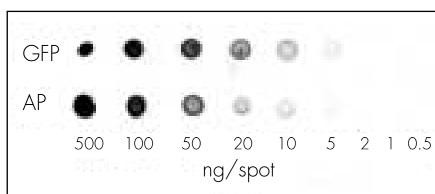
Strep-tag® HRP Detection Kit

for a fast detection of *Strep*-tag II proteins in Western blots or ELISA





Direct detection of recombinant *Strep*-tag II GFP (Green Fluorescent Protein) in a Western blot using *Strep*-Tactin HRP conjugate.



Direct detection of the recombinant *Strep*-tag II proteins GFP (MW 28 kDa) and *E. coli* alkaline phosphatase (monomer 48.5 kDa) in a dot blot using *Strep*-Tactin HRP conjugate.

The *Strep*-tag HRP Detection Kit

As positive control in Western blots use the *Strep*-tag Protein Ladder (page 32)

The *Strep*-tag HRP Detection Kit is used to detect *Strep*-tag II fusion proteins transferred to a blotting membrane. The ready-to-use system includes all buffers and reagents required for blocking, washing and the chromogenic reaction on a total of 1000 cm² membrane. Please note that the *Strep*-Tactin® HRP (horse radish peroxidase) conjugate included in the kit is also available separately.

Kit components (sufficient for 1000 cm² membrane)

Component	Description	Amount
Control protein	Recombinant *Strep*-tag II fusion protein	24 µg
Buffer SI	Blocking and incubation buffer 10 x	50 ml
Buffer W	Washing buffer 20 x	2 x 50 ml
Strep-Tactin HRP conjugate	at least 2 mol HRP / mol *Strep*-Tactin	0.125 ml
Buffer ER	HRP reaction buffer 10 x	25 ml
HRP substrate	30% H_2O_2	0.5 ml
Chromogen	3% 4–chloro naphtol in methanol	3.0 ml

Specifications of *Strep*-Tactin HRP conjugate

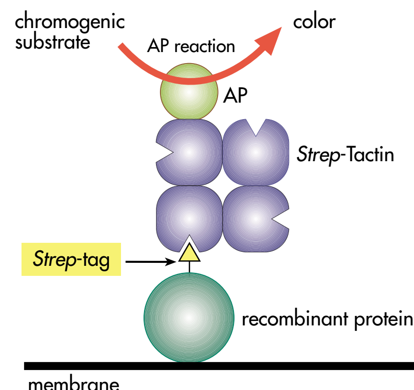
Detection	N- or C-terminal or internal *Strep*-tag II
Sensitivity in dot blots	> 7 ng/dot
Sensitivity in Western blots	> 7 ng/band
Cross reactivity	Reacts with Biotin Carboxyl Carrier Protein (BCCP, 22.5 kDa) in *E. coli* extracts. Generally, biotinylated proteins can be sufficiently masked by a 10 minute pre-incubation with Biotin Blocking Buffer (page 28).
Form	Suspended in 0.01 M phospate buffered saline pH 7.4 containing BSA and 0.01 % Thimerosal as preservative. Can be diluted 1:10,000 for detection of *Strep*-tag II fusion proteins bound to microplates or 1:4,000 for detection of *Step*-tag II fusion proteins after Western blotting.
Stability	1 year
Storage	4 °C
Shipment	RT

product	amount	cat. no.
Strep-tag HRP Detection Kit	1 kit	2-1502-000
Strep-Tactin HRP conjugate	0.5 ml	2-1502-001
Strep-tag Protein Ladder (lyophilized) (see page 32)	240 µg; 100 applications	2-1011-100

Strep-tag® AP Detection Kit

for a fast detection of *Strep*-tag II proteins in Western blots

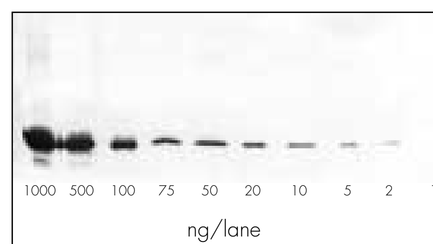
The *Strep*-tag AP Detection Kit is used to detect *Strep*-tag fusion proteins transferred to a blotting membrane. The ready-to-use system includes all buffers and reagents required for blocking, washing and the chromogenic reaction on a total of 1000 cm² membrane. Please note, that the *Strep*-Tactin® AP (alkaline phosphatase) conjugate included in the kit is also available separately.



Kit components (sufficient for 1000 cm² membrane)

Component	Description	Amount
Control protein	Recombinant *Strep*-tag II fusion protein	24 µg
Buffer SI	Blocking and incubation buffer 10 x	50 ml
Buffer W	Washing buffer 20 x	60 ml
Strep-Tactin AP conjugate	at least 2 mol AP / mol *Strep*-Tactin	0.125 ml
Buffer ER	AP reaction buffer 10 x	25 ml
AP substrate	BCIP* (5-bromo-4-chloro-3-indolylphosphate p-toluidine salt)	1 ml
Chromogen	NBT* (nitroblue tetrazolium chloride)	1 ml

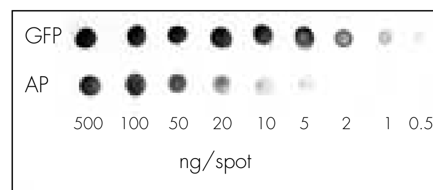
*Store BCIP and NBT solutions immediately at - 20 °C upon arrival.
All other solutions can be stored at 4-8 °C until use. Shipment at RT.



Direct detection of recombinant *Strep*-tag II GFP (Green Fluorescent Protein) in a Western blot using *Strep*-Tactin AP conjugate.

Specifications of *Strep*-Tactin AP conjugate

Alkaline Phosphatase	Calf intestine
Detection	N- or C-terminal or internal *Strep*-tag II
Sensitivity in dot blots	> 1 ng/dot
Sensitivity in Western blots	> 2 ng/band
Cross reactivity	Reacts with Biotin Carboxyl Carrier Protein (BCCP, 22.5 kDa) in *E. coli* extracts. Generally, biotinylated proteins can be sufficiently masked by a 10 minute pre-incubation with Biotin Blocking Buffer (page 28).
Form	Suspended in 0.01 M phospate buffered saline pH 7.4 containing BSA and 0.01 % Thimerosal as preservative. Can be diluted 1:4,000 for detection of *Strep*-tag II fusion proteins after Western blotting. (Please note that for ELISA we recommend to use the *Strep*-Tactin HRP conjugate mentioned on page 30).
Stability	1 year
Storage	4 °C
Shipment	RT

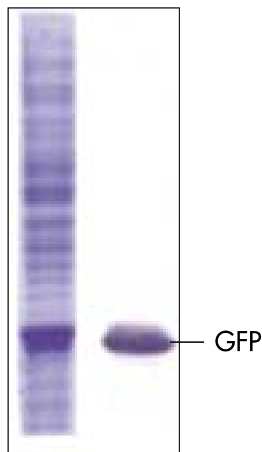


Direct detection of the recombinant *Strep*-tag II proteins GFP (MW 28 kDa) and *E. coli* alkaline phosphatase (monomer 48.5 kDa) in a dot blot using *Strep*-Tactin AP conjugate.

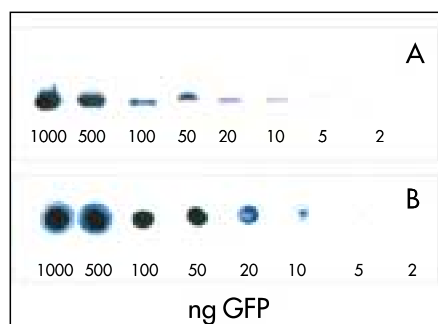
product	amount	cat. no.
Strep-tag AP Detection Kit	1 kit	2-1503-000
Strep-Tactin AP conjugate	0.5 ml	2-1503-001
Strep-tag Protein Ladder (lyophilized) (see page 32)	240 µg; 100 applications	2-1011-100

The *Strep*-tag AP Detection Kit

As positive control in Western blots use the *Strep*-tag Protein Ladder (page 32)



Highly specific detection of recombinant *Strep*-tag proteins in *E.coli* lysates by *Strep*-tag II specific monoclonal antibody. Left, Coomassie stained SDS PAGE of Green Fluorescent Protein (GFP) crude lysate; right, corresponding Western Blot. Note: Biotin Carboxy Carrier Protein (BCCP) was not detected.



Direct detection of the recombinant *Strep*-tag II GFP in Western blot and dot blot using *Strep*-tag II specific monoclonal antibody and rabbit anti-mouse IgG HRP conjugate as detection system. A = Western blot, B = dot blot.

Strep-tag® II specific monoclonal antibody

New

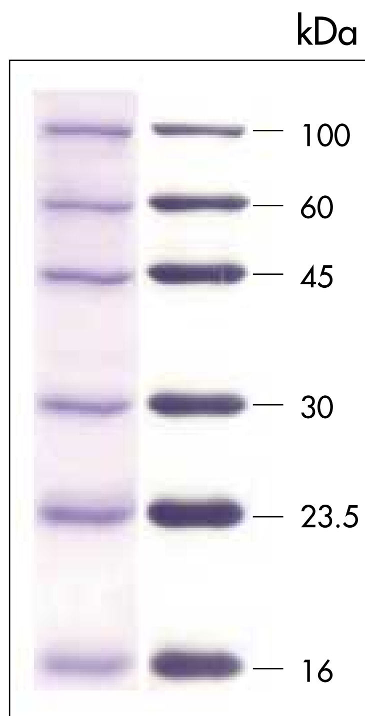
A new detection tool for recombinant *Strep*-tag proteins

The *Strep*-tag II specific monoclonal antibody is highly selective and shows, therefore, a very low background.

Specifications of *Strep*-tag II specific monoclonal antibody

Host animal	Mouse
Subclass	IgG1
Specifity	*Strep*-tag II
Detection	N- or C-terminal or internal *Strep*-tag II
Sensitivity in Western blots	5 ng/lane
Sensitivity in dot blots	5 ng/dot
Cross reactivity	No cross reactivity known
Form	Lyophilized from NH_4HCO_3
Stability	2 years
Storage	-20°C
Shipment	RT

product	amount	cat. no.
Strep-tag II specific monoclonal antibody		
purified	100 µg	2-1507-001
crude cell culture supernatant, lyophilized	for 25 Western blots	2-1508-025
crude cell culture supernatant, lyophilized	for 50 Western blots	2-1508-050



gels and blots can be compared

Strep-tag Protein Ladder

New

Accurate MW determination and positive control on Western blots!

The new *Strep*-tag Protein Ladder simplifies the comparison of Coomassie stained gels with corresponding Western blots. Thus, *Strep*-tag fusion proteins can be located accurately in complex band patterns on Coomassie gels.

The *Strep*-tag Protein Ladder is a mixture of six recombinant, highly purified *Strep*-tag proteins employed for precise sizing of proteins by SDS-PAGE. The proteins resolve into clearly identifiable sharp and evenly stained bands from 16 to 100 kDa when analyzed on a SDS gel and stained with Coomassie Blue.

As each protein contains the *Strep*-tag II sequence which is detected by *Strep*-Tactin® conjugates or the *Strep*-tag II specific monoclonal antibody, the ladder can also be used for MW determinations on Western blots and serves as a positive control for the various detection systems (see above and pages 29-31).

product	amount	cat. no.
Strep-tag Protein Ladder (lyophilized)	240 µg; 100 applications	2-1011-100

For prices see separate price list or www.iba-go.com • info@iba-go.com

Strep-tag® assay system

Strep-Tactin® coated microplates

Fast, selective and sensitive assay for 8 to 96 samples

Ready-to-use *Strep*-Tactin coated 8-well strips provide the power of our *Strep*-tag system in a solid-phase, multi-well format for convenient assays and high throughput screenings of biomolecules tagged with *Strep*-tag II. The **strips are supplied framed** in sets of 12, resulting in a 96-well configuration compatible with standard multichannel pipettes and automated plate washers and plate readers.

Microplate consisting of twelve 8-well strips coated with *Strep*-Tactin.

Features and benefits

- Oriented immobilization of recombinant proteins with N-terminal, C-terminal or internal *Strep*-tag II
- Effective screening procedures
- Minimal non-specific binding
- High and constant activity
- Minimal coefficients of variation (cv; see page 34)

Principle and properties

The *Strep*-tag assay system uses 8-well strips coated with *Strep*-Tactin, allowing *Strep*-tag fusion proteins to be selectively captured from complex mixtures of molecules. The biomolecules are presented to interacting partners in a uniform manner which results in reliable and reproducible assay formats.

Procedure

Strep-Tactin coated microplates (12 x 8-well strips) are offered freeze dried and ready-to-use. Solutions containing the *Strep*-tag II fusion protein can be applied without any prior wetting step.

Applications

- Antibody or serum screening (see Figure on page 34, top)
- Diagnostic assays
- Expression cloning
- Protein interaction studies
- Screening of engineered enzymes
- Drug screening

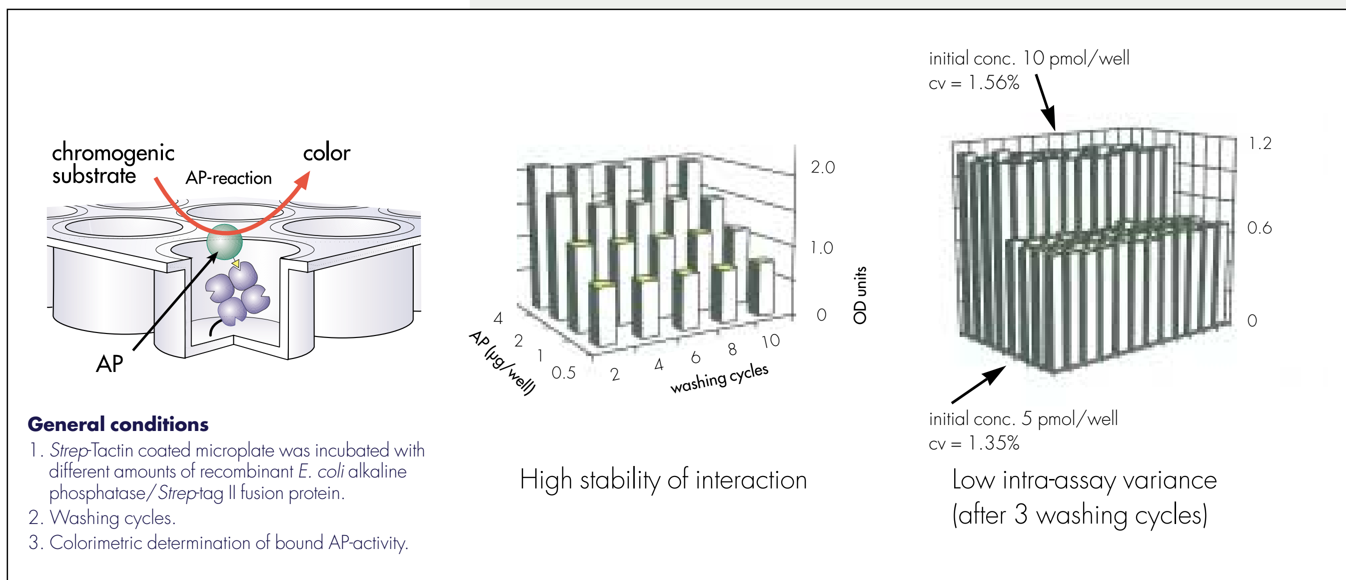
Specifications of *Strep*-Tactin coated microplates

Strep-tag fusion protein binding capacity	100 ng *Strep*-tag II fusion protein/ well
Intra- and inter-assay variance (cv)	< 1.6% and < 3.0%, respectively
Max. volume per well	300 µl
Optimal pH	8.0
Stability	> 1 year at 4 °C
Storage	4 °C
Shipment	RT

product	amount	cat. no.
Strep-Tactin coated microplates	1 plate	2-1501-001
(12 x 8 wells)	5 plates	2-1501-005

Capture of functional target proteins on *Strep*-Tactin® coated microplates

Determination of an alkaline phosphatase (AP) *Strep*-tag® II fusion protein



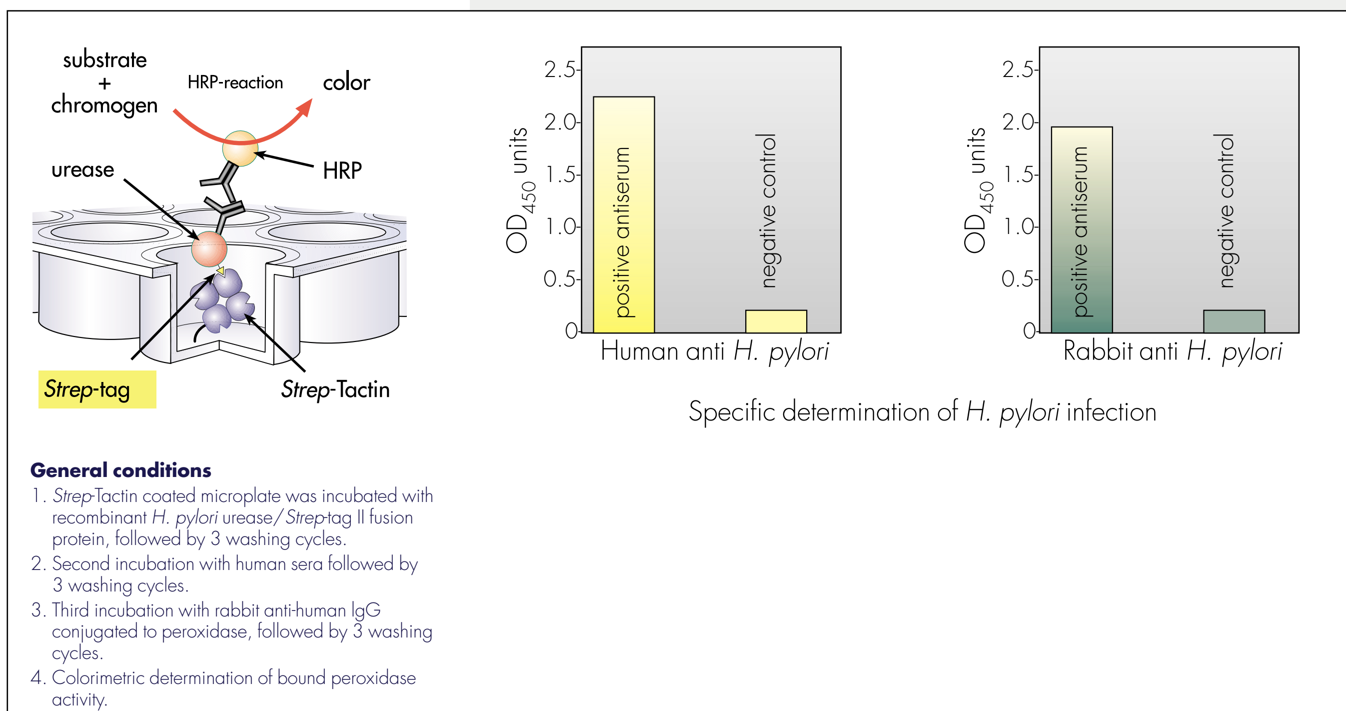
General conditions

1. *Strep*-Tactin coated microplate was incubated with different amounts of recombinant *E. coli* alkaline phosphatase/*Strep*-tag II fusion protein.
2. Washing cycles.
3. Colorimetric determination of bound AP-activity.

High stability of interaction

Low intra-assay variance (after 3 washing cycles)

H. pylori urease as antigen in a solid phase immunoassay



General conditions

1. *Strep*-Tactin coated microplate was incubated with recombinant *H. pylori* urease/*Strep*-tag II fusion protein, followed by 3 washing cycles.
2. Second incubation with human sera followed by 3 washing cycles.
3. Third incubation with rabbit anti-human IgG conjugated to peroxidase, followed by 3 washing cycles.
4. Colorimetric determination of bound peroxidase activity.

Specific determination of *H. pylori* infection

For prices see separate price list or www.iba-go.com • info@iba-go.com

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A) Articles of general interest - reviews

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Double tag, *Strep*-tag/6xHis-tag

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D) Detection

53. Kleymann G, Ostermeier C, Heitmann K, Haase W, Michel H, 1995: The Journal of Histochemistry and Cytochemistry 43: 607-614. Use of antibody fragments (Fv) in immunochemistry.
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6xHis-tag and Ni-NTA technology

The optimal partner for *Strep*-tag® in double tag proteins – solving your protein purification problem!

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Purification of full length proteins 38
Highest purification factors 38
Using denaturing OR physiological purification conditions 38
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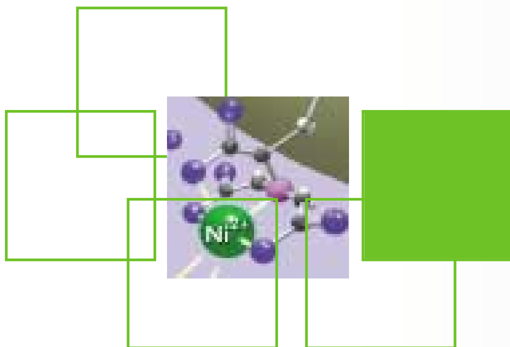


6xHis-tag protein binding to Ni-NTA resin.

Introduction

Protein expression is a complex topic with many variables. Therefore, it is e.g. hard to predict whether a recombinant protein is expressed soluble or forms inclusion bodies or is partially degraded. To be prepared for the most common difficulties (see below) the attachment of two tags to a recombinant protein provides the flexibility to obtain a highly pure and homogenous protein preparation.

Important reasons for two different affinity tags on one protein are

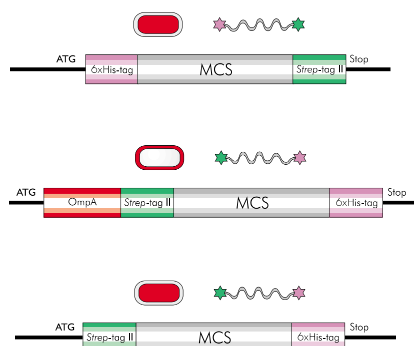
- Purification of full length proteins
- Highest purification factors
- Using denaturing OR physiological purification conditions
- Optimizing purification protocols directly from the culture medium

A smart double tag pair is the combination of *Strep*-tag® and 6xHis-tag. Generally, it is recommended to attach one tag to the N-terminus and the other to the C-terminus.

Purification of full length proteins

Recombinant proteins may be partially degraded during expression which cannot be prevented by adding protease inhibitors during downstream processing. Soluble degradation products still carrying the tag are co-purified and cause an inhomogeneous protein preparation with protein fragments of different lengths. This problem can be solved by adding a second tag to the other protein terminus. Performing a second purification run using the affinity of this second tag selects for full-length proteins.

Reference:
Fiedler M, Horn C, Bandtlow C, Schwab ME, Skerra A, 2002: Protein Eng. Nov;15(11):931-41.
An engineered IN-1 F(ab) fragment with improved affinity for the Nogo-A axonal growth inhibitor permits immunochemical detection and shows enhanced neutralizing activity.



Highest purification factors

Strep-tag purification enables isolation of recombinant protein at over 99% purity in one step under physiological conditions (see page 22). However, depending on the recombinant protein, impurities may arise. Although the problem may be solved by changing the resin (see page 20), the usage of a second affinity tag may potentially be more efficient. To obtain reliable results in any downstream application like, e.g., crystallography, immunizations or HTS, a highly pure product is essential.

Using denaturing OR physiological purification conditions

It is hard to predict whether a recombinant protein is expressed soluble or forms inclusion bodies. This depends e.g. on the strength of the promoter used for expression and on the folding rates of the protein itself. If the protein is soluble and functional, *Strep*-tag is the best choice because of its mild purification conditions keeping the protein bioactive. If the protein is insoluble and forms inclusion bodies after expression, it has to be solubilized with chaotropic salts like guanidine or urea at high concentrations in order to allow affinity chromatography. Purification under such conditions can be excellently achieved by using the metal chelate activity of the 6xHis-tag. Proteins can be purified and optionally be refolded on Ni-NTA columns. If necessary, they can be further purified as refolded protein via *Strep*-tag in physiological buffer.

Double tag vectors with both, *Strep*-tag and 6xHis-tag, are described on pages 44, 45.

For prices see separate price list or www.iba-go.com • info@iba-go.com

Optimizing purification directly from the culture medium

Especially for higher expression hosts (e.g. insect cells) an often applied expression strategy is the secretion of the recombinant protein to the culture medium. In such cases a first capturing step with the 6xHis-tag is recommended. Due to the high affinity of the 6xHis-tag to Ni-NTA matrix, the recombinant protein can be efficiently collected even by performing batch purification. As a positive side-effect, biotin, which is incompatible with *Strep*-tag purification and generally present in culture media, is removed in this step. Thus, if necessary, a second purification step can be performed using *Strep*-tag leading to highly pure protein.

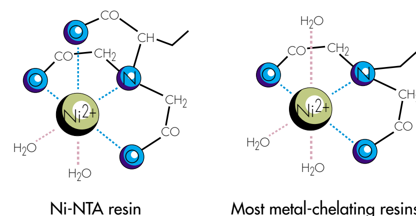
For the new *Strep*/His Starter Kit for purification of double tag proteins see page 40.

Ni-NTA protein purification system

Recombinant proteins containing a 6xHis-tag can be purified by Ni-NTA (nickel-nitrilotriacetic acid) chromatography which is based on the interaction between a transition Ni^{2+} ion immobilized on a matrix and the histidine side chains. Following washing of the matrix 6xHis-tag fusion proteins can be eluted by adding free imidazole or EDTA or by reducing the pH.

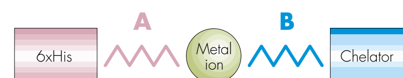
IBA is now offering several new Ni-NTA resins, columns and cartridges for purification of 6xHis-tag proteins.

Tighter binding of nickel ions with NTA



Comparison of the interactions of different metal-chelating resins with nickel ions.

Tighter binding of the 6xHis tag



The capture of 6xHis-tag proteins by metal-chelate affinity matrices relies on two interactions. Both are important for optimal performance. If interaction A is weak, there is no bindig of the 6xHis-tag protein. If interaction A is strong, but interaction B weak, protein is lost as protein-metal complexes during wash steps. When NTA ligand and nickel are used to bind 6xHis-tag molecules, both interactions are stronger, providing advantages over systems that rely on other ligands or metals.

Features

- One-step purification from crude lysates
- High binding affinity and high capacity
- Purification under native or denaturing conditions is possible
- Pre-charged, ready-to-use matrices for any scale of purification

Ni-NTA purification resins and columns

Ni-NTA Sepharose resin and columns

New

For purification of 6xHis-tag proteins by gravity flow chromatography

Ni-NTA Sepharose offers high binding capacity and minimal non-specific binding. This material has excellent handling properties for most scales of batch and column purification. Pre-packed columns are available in different formats (1 and 5 ml bed volume).

Specifications Ni-NTA Sepharose

Binding capacity	5-10 mg/ml (300-500 nmol of a 20 kDa protein)
Support	Sepharose CL-6B
Bead structure	Cross-linked, 6% agarose
Bead size	45-165 µm
Maximal pressure	2.8 psi
Form (bulk)	50% suspension in 30% ethanol, pre-charged with Ni^{2+}

product	amount	cat. no.
Ni-NTA Sepharose gravity flow column (1 ml bed volume)	1 column	2-3202-001
Ni-NTA Sepharose gravity flow column (5 ml bed volume)	1 column	2-3202-051
Ni-NTA Sepharose	20 ml 50 % suspension (= 10 ml column bed volume)	2-3201-010

Ni-NTA resin is manufactured by Qiagen

Column formats are illustrated on page 21.

Cartridges with 1 ml or 5 ml Ni-NTA Superflow for low pressure chromatography. Cartridges can be connected in series to enlarge capacity. They can be used with syringes. Adapters are available for use with peristaltic pump chromatography systems or FPLC / HPLC workstations (see page 21). The cartridges have a female luer lock inlet and a male luer lock outlet.

Further column formats are illustrated on page 21.

Ni-NTA Superflow resin and columns

New

For purification of 6xHis-tag proteins by gravity flow and FPLC

Ni-NTA Superflow combines superior mechanical stability and outstanding flow characteristics with high dynamic capacity. This resin allows purification of 6xHis-tag proteins under gravity flow as well as high flow rates and pressures for efficient FPLC applications. Pre-packed columns for gravity flow as well as pre-packed cartridges for low pressure chromatography by means of syringes, peristaltic pump systems or FPLC/HPLC workstations (see page 21) are available in 1 and 5 ml formats.

Specifications Ni-NTA Superflow

Binding capacity	5-10 mg/ml (300-500 nmol of a 20 kDa protein)
Support	Superflow
Bead structure	Highly cross-linked, 6% agarose
Bead size	60-160 µm
Maximal pressure	140 psi
Form (bulk)	50% suspension in 30% ethanol, pre-charged with Ni^{2+}

product	amount	cat. no.
Ni-NTA Superflow gravity flow column (1 ml bed volume)	1	2-3207-001
Ni-NTA Superflow gravity flow column (5 ml bed volume)	1	2-3207-051
Ni-NTA Superflow cartridge (1 ml bed volume)	1 cartridge	2-3211-001
	5 cartridges	2-3211-005
Ni-NTA Superflow cartridge (5 ml bed volume)	1 cartridge	2-3212-001
	5 cartridges	2-3212-005
Ni-NTA Superflow 50 % suspension	20 ml (= 10 ml column bed volume)	2-3206-010

Ni-NTA resin is manufactured by Qiagen

Strep/His Starter Kit

Further Starter Kits can be found on pages 19, 53.

Strep/His Starter Kit

New

for purification of double tag proteins

This unique new Starter Kit contains all reagents essential for the native purification of a double tag protein containing *Strep*-tag and 6xHis-tag. The first purification is performed on a Ni-NTA cartridge (see also page 39) while the second purification uses a *Strep*-Tactin cartridge (see pages 21, 23, 24), selecting for 6xHis-tag and *Strep*-tag, respectively.

Kit contents

- 1 *Strep*-Tactin Superflow cartridge (1 ml)
- 1 Ni-NTA Superflow cartridge (1 ml)
- *Strep*-Tactin Washing Buffer ("Buffer W")
- *Strep*-Tactin Elution Buffer ("Buffer E")
- *Strep*-Tactin Regeneration Buffer ("Buffer R")
- Ni-NTA Equilibration Buffer ("Lysis Buffer")
- Ni-NTA Washing Buffer ("Wash Buffer")
- Ni-NTA Elution Buffer ("Elution Buffer")
- $NiSO_4$ * $6H_2O$
- Anhydrotetracycline for induction of expression
- Control plasmid with 38 kDa protein insert
- *Strep*-Tactin HRP conjugate for Western blot detection
- Comprehensive Manual

cat. no. 2-1107-000

Patent and licensing information see page 121.

Vectors

with *Strep*-tag® and/or 6xHis-tag

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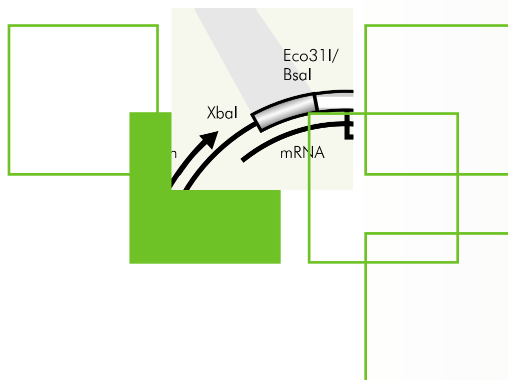
Overview of *Strep*-tag and double tag vectors 45

Overview of 6xHis-tag vectors 46

Remarks on protein expression 47

Cloning system and Primer D'Signer 48



Expression systems Tet and T7

IBA offers the Tet and T7 expression systems producing recombinant proteins in *E. coli*. Therefore, a variety of vectors with unique characteristics is available supporting you with the purification of your protein of interest. The choice of system and vector depends on your protein and its characteristics.

pASK-IBA vector maps see pages 45, 46

Features and benefits of the Tet expression system

- High-level expression in *E. coli* with pASK-IBA vectors
- Tightly regulated expression due to the tet promoter
- Enhanced stability of cytotoxic genes
- Universal cloning strategy with one restriction enzyme (see pages 48, 49)
- N- or C-terminal *Strep*-tag II fusion
- N- or C-terminal 6xHis-tag fusion
- *Strep*-tag II/6xHis-tag – double tag vectors
- Cytosolic or periplasmic expression
- Inexpensive induction with anhydrotetracycline (see below)
- Ampicillin or chloramphenicol resistance

Reference:
Skerra, A. (1994). Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in *Escherichia coli*. Gene, 151, 131-135.

Principle and properties

pASK-IBA vectors work with the tightly regulated tet promoter. The tet repressor is encoded on the pASK-IBA plasmids and is constitutively expressed from the β-lactamase or the chloramphenicol acetyl transferase promoter, respectively. This special arrangement guarantees a **balanced stochiometry between repressor molecules and plasmid copy number**. Expression of the foreign gene is stringently repressed until efficient chemical induction with a low concentration of **anhydro-tetracycline**. In contrast to the lac promoter – which is leaky, susceptible to catabolite repression (cAMP-level, metabolic state), and influenced by chromosomally encoded repressor molecules – the tetA promoter/operator is **tightly controlled and not functionally coupled to any cellular regulation mechanisms or genetic background.**

As a consequence, special *E. coli* strains or extra plasmids and are not required a broad range of culture media and conditions can be used. For example, glucose minimal media and even the XL1-Blue bacterial strain, which carries an episomal copy of the tetracycline resistance gene, can be used for expression. The pASK-IBA expression system is **stable under many conditions**, including **fermentation**, and is **easy-to-handle**.

Further elements of the vectors are a tandem ribosome binding site (RBS) which ensures efficient initiation of translation, the strong terminator of the lipoprotein gene in order to prevent read-through, the intergenic region of the bacteriophage f1 which provides a means for preparing ssDNA and a β-lactamase or chlor-amphenicol acetyl transferase gene*. The vectors do not mediate resistance against tetracycline.

The following *E. coli* strains have already been used successfully for Tet expression with our pASK-IBA vectors: JM83, WK6, B, BL21, MG1655, W3110, BL21(DE3), BLR(DE3), XL1-Blue, BL21-CodonPlus™-RIL

For secretion, we recommend JM83. For cytoplasmic expression *E. coli* B strains are recommended, since they lack the lon protease and the ompT outer membrane protease that can degrade proteins during purification (Grodberg and Dunn, 1988, J.Bacteriol. 170, 1245).

Please note that we are not aware of an *E. coli* strain that is incompatible with the Tet expression system.

product	amount	cat. no.
Anhydrotetracycline	50 mg (for 250 liter *E.coli* culture)	2-0401-001
Anhydrotetracycline	25 mg	2-0401-002

Literature for anhydrotetracycline:
Degenkolb J, Takahashi M, Ellestad GA, Hillen W, 1991:Antimicrob. Agents Chemother. 35, No 8, 1591-1595. Structural requirements of tetracycline-Tet repressor interaction: Determination of equilibrium binding constants for tetracycline analogues with the Tet repressor.

*Using cloning vectors with β-lactamase resistance gene may be associated with some limitations since ampicillin is degraded quite fast in bacterial culture medium. Therefore, we are now offering our *Strep*-tag II vectors pASK-IBA2C to pASK-IBA7C with chloramphenicol resistance instead of ampicillin resistance.

Features and benefits of the T7 expression system

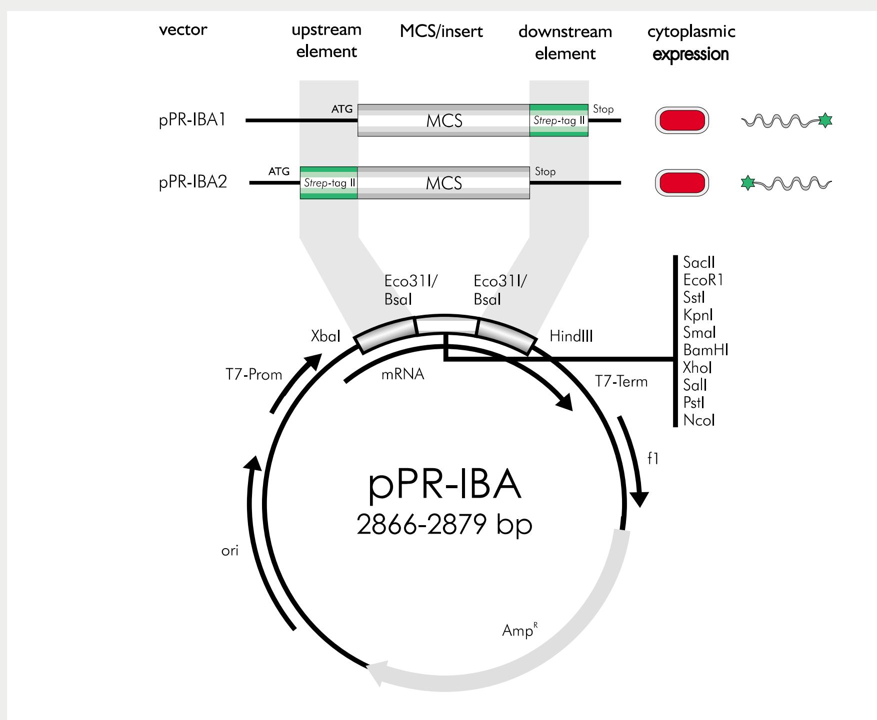
- High-level expression by bacteriophage T7 promoter
- High-level transcription by T7 RNA polymerase in BL21 strains
- High-level expression of non-toxic proteins
- Induction by IPTG
- N- or C-terminal *Strep*-tag II
- Suitable for *in vitro* transcription/translation

Principle and properties

The Tet promoter is of medium strength which leads to high level expression of certain proteins depending e.g. on their folding rate and stability - characteristics which can hardly be predicted. Some proteins, however, can only be expressed at high level if transcribed by a stronger promoter. In such cases we recommend the use of the T7 promoter.

The T7 expression system is encoded on the pPR-IBA vectors. The system uses the T7 promoter and T7 RNA polymerase for high-level transcription of the gene of interest. Expression of the target genes is induced by providing a source of T7 RNA polymerase in the host cell. This is accomplished by using *E. coli* BL21 which contains a chromosomal copy of the T7 RNA polymerase gene. The latter is under control of the lacUV5 promoter which can be induced by IPTG.

pPR-IBA vectors (T7 promoter)



For Multiple Cloning Sites (MCS) please refer to the Appendix.

The primers created for cloning into pPR-IBA1 and pPR-IBA2 are also compatible with our pASK-IBA expression vectors (see also pages 44-46):

- pPR-IBA1 cloning primers are suitable for pASK-IBA3 and pASK-IBA33
- pPR-IBA2 cloning primers are suitable for pASK-IBA4, pASK-IBA5 and pASK-IBA35

Sequencing primers are described on page 44.

We also offer custom synthesis of primers (see page 75).

Comparison of all pASK-IBA and pPR-IBA Vectors

IBA is offering several different vectors with varying properties

Plasmid	*Strep*-tag	6xHis-tag	Secretion	Removal of the tag	protease	resistance	amount	cat. no.
pASK-IBA2	C-terminal	-	Yes	No	not applicable	Amp	5 µg	2-1301-000
pASK-IBA2C	C-terminal	-	Yes	No	not applicable	CAT	5 µg	2-1321-000
pASK-IBA3	C-terminal	-	No	No	not applicable	Amp	5 µg	2-1302-000
pASK-IBA3C	C-terminal	-	No	No	not applicable	CAT	5 µg	2-1322-000
pASK-IBA4	N-terminal	-	Yes	No	not applicable	Amp	5 µg	2-1303-000
pASK-IBA4C	N-terminal	-	Yes	No	not applicable	CAT	5 µg	2-1323-000
pASK-IBA5	N-terminal	-	No	No	not applicable	Amp	5 µg	2-1304-000
pASK-IBA5C	N-terminal	-	No	No	not applicable	CAT	5 µg	2-1324-000
pASK-IBA6	N-terminal	-	Yes	Yes	factor Xa	Amp	5 µg	2-1305-000
pASK-IBA6C	N-terminal	-	Yes	Yes	factor Xa	CAT	5 µg	2-1325-000
pASK-IBA7	N-terminal	-	No	Yes	factor Xa	Amp	5 µg	2-1306-000
pASK-IBA7C	N-terminal	-	No	Yes	factor Xa	CAT	5 µg	2-1326-000
pASK-IBA12	N-terminal	-	Yes	Yes	thrombin	Amp	5 µg	2-1311-000
pASK-IBA13	N-terminal	-	No	Yes	thrombin	Amp	5 µg	2-1312-000
pASK-IBA14	N-terminal	-	Yes	Yes	enterokinase	Amp	5 µg	2-1313-000
pASK-IBA15	N-terminal	-	No	Yes	enterokinase	Amp	5 µg	2-1314-000
pASK-IBA32	-	C-terminal	Yes	No	not applicable	Amp	5 µg	2-1332-000
pASK-IBA33	-	C-terminal	No	No	not applicable	Amp	5 µg	2-1333-000
pASK-IBA35	-	N-terminal	No	No	not applicable	Amp	5 µg	2-1335-000
pASK-IBA37	-	N-terminal	No	Yes	factor Xa	Amp	5 µg	2-1337-000
pASK-IBA43	C-terminal	N-terminal	No	No	not applicable	Amp	5 µg	2-1343-000
pASK-IBA44	N-terminal	C-terminal	Yes	No	not applicable	Amp	5 µg	2-1344-000
pASK-IBA45	N-terminal	C-terminal	No	No	not applicable	Amp	5 µg	2-1345-000
pPR-IBA1	C-terminal	-	No	No	not applicable	Amp	5 µg	2-1390-000
pPR-IBA2	N-terminal	-	No	No	not applicable	Amp	5 µg	2-1391-000

Special offer: 3 vectors of choice for the price of two! cat no. 2-1300-000

For vector maps please refer to pages 43, 45, 46.
The Multiple Cloning Sites (MCS) can be found in the Appendix.

Sequencing primers

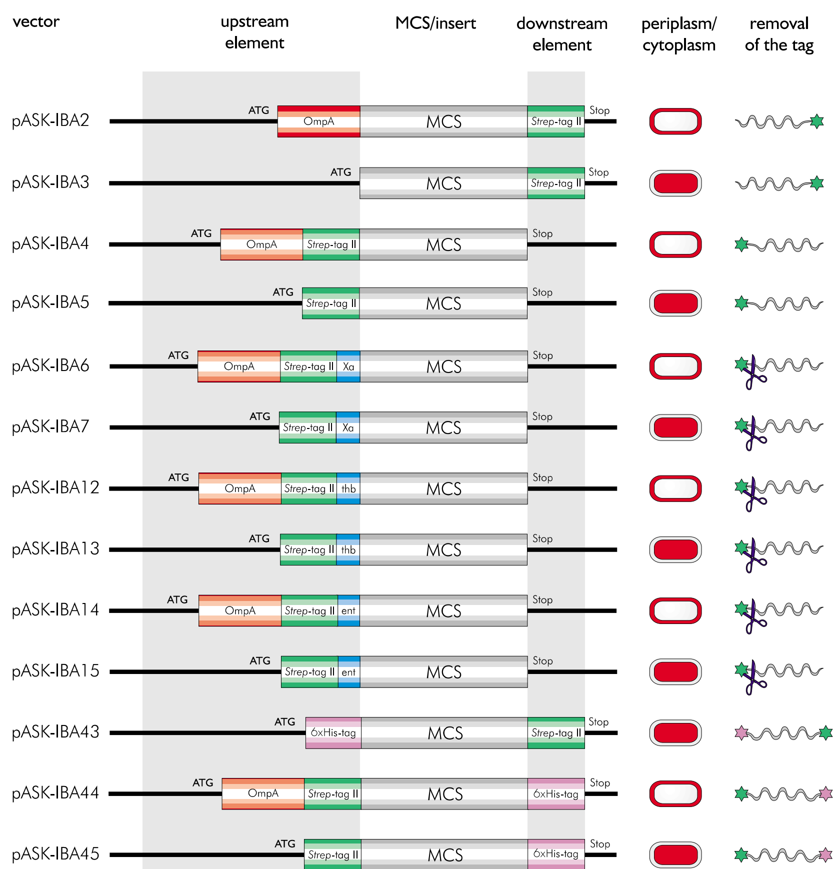
product	amount	cat. no.
Forward sequencing primer for pASK-IBA vectors	1nmol, 10 pmol/µl HPLC purified	5-0000-101
Reverse sequencing primer for pASK-IBA vectors	1 nmol, 10 pmol/µl HPLC purified	5-0000-102
Forward and reverse sequencing primers for pASK-IBA vectors	1 nmol each, 10 pmol/µl HPLC purified	5-0000-103
Forward sequencing primer for pPR-IBA vectors (T7 primer)	1 nmol, 10 pmol/µl HPLC purified	5-0000-111
Reverse sequencing primer for pPR-IBA vectors (T7 primer)	1 nmol, 10 pmol/µl HPLC purified	5-0000-112
Forward and reverse sequencing primer for pPR-IBA vectors (T7 primer)	1 nmol each, 10 pmol/µl HPLC purified	5-0000-113

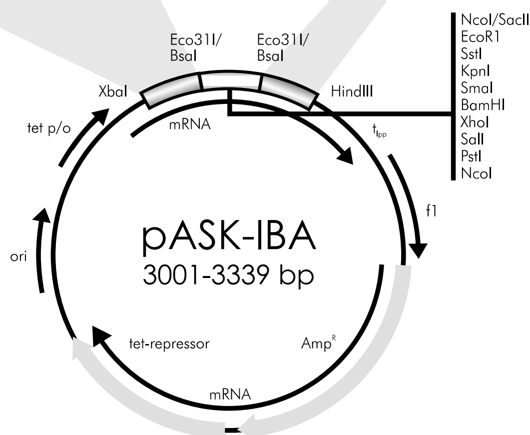
Overview of *Strep*-tag® and double tag vectors

In addition to Strep-tag vectors, we are now also offering double tag vectors with both, *Strep*-tag and 6xHis-tag. Two different affinity tags on a protein yields highest purification factors and allows purification of full-length proteins under denaturing or physiological conditions. Also, purification protocols directly from the culture medium can be optimized. For details please refer to page 37 ff.

Except for the antibiotic resistance genes (Amp^R, Cam^R) the pASK-IBA vectors differ only in between the *Xba*I and *Hind*III restriction sites.

For details on double tag proteins please refer to page 37 ff.





Structure of a selection of pASK-IBA expression vectors with *Strep*-tag or *Strep*-tag and 6xHis-tag. pASK-IBA2 to pASK-IBA7 are also available with chloramphenicol resistance instead of ampicillin resistance. MCS, f1, Amp^R, , ori, t_{lpp} , ompA, Xa, thb, ent, and tetR denote the multiple cloning site, intergenic region of phage f1, β-lactamase gene, origin of replication of the pUC family of plasmids, lpp terminator of transcription, ompA signal sequence, factor Xa recognition sequence, thrombin recognition sequence, enterokinase recognition sequence and tet repressor gene, respectively. The expression cassette is under transcriptional control of the tetA promoter/operator. The tet repressor gene has been placed as a second cistron immediately behind the cistron encoding ß-lactamase and is thus under transcriptional control of the constitutive ß-lactamase promoter.

Digesting the vector with one of the type IIS restriction enzymes *Bsa*I, *Eco*31I, or *Bsm*FI leads to the generation of defined 5' overhangs in the upstream and downstream elements, which are not mutually compatible and can therefore not re-ligate. Upstream elements may be DNA sequences encoding the OmpA signal peptide, an initiator methionine, *Strep*-tag II, 6xHis-tag or the protease recognition sequence, while downstream elements may be the *Strep*-tag II, 6xHis-tag or a stop codon. The gene to be cloned must be equipped with compatible overhangs, e.g. by incorporation of appropriate restriction sites by PCR and subsequent cleavage (see pages 48, 49).

Order information see page 44.

References:

Skerra A, Schmidt TGM, 2000: Meth. Enzymol. 326: 271-304. Use of the *Strep*-tag and streptavidin for recombinant protein purification and detection.

Skerra A, 1994: Gene 151: 131-135. Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in *Escherichia coli*.

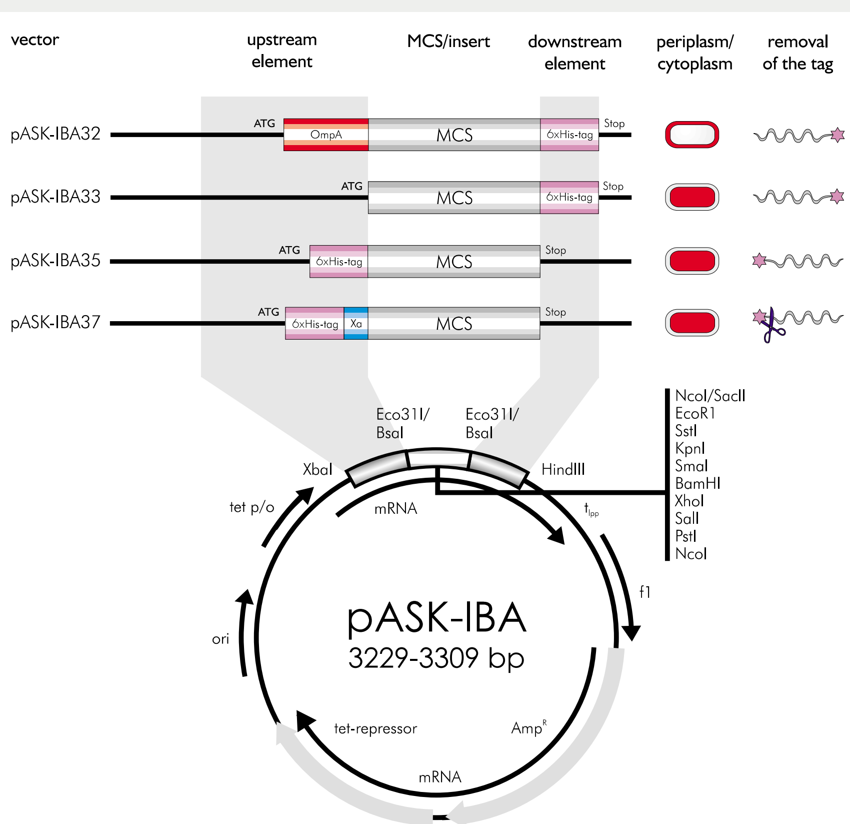
For Multiple Cloning Sites (MCS) please refer to the Appendix.

Overview of 6xHis-tag vectors

The pASK-IBA vectors containing His-tag differ only in between the *Xba*I and *Hind* III restriction sites.

Structure of a selection of pASK-IBA expression vectors with 6xHis-tag. MCS, f1, Amp^R, ori, t_{lpp} , ompA, Xa, and tetR denote the multiple cloning site, intergenic region of phage f1, β-lactamase gene, origin of replication of the pUC family of plasmids, lpp terminator of transcription, ompA signal sequence, factor Xa recognition sequence, and tet repressor gene, respectively. The expression cassette is under transcriptional control of the tetA promoter/ operator. The tet repressor gene has been placed as a second cistron immediately behind the cistron encoding ß-lactamase and is thus under transcriptional control of the constitutive ß-lactamase promoter.
Digesting the vector with one of the type IIS restriction enzymes *Bsa*I, *Eco*31I, or *Bsm*FI leads to the generation of defined 5' overhangs in the upstream and downstream elements, which are not mutually compatible and can therefore not re-ligate. Upstream elements may be DNA sequences encoding the OmpA signal peptide, an initiator methionine, 6xHis-tag or the protease recognition sequence, while downstream elements may be the 6xHis-tag or a stop codon. The gene to be cloned must be equipped with compatible overhangs, e.g. by incorporation of appropriate restriction sites by PCR and subsequent cleavage (see pages 48, 49).

Order information see page 44.



For Multiple Cloning Sites (MCS) please refer to the Appendix.

Free Primer D'Signer software: see www.iba-go.com and page 48.

Patent and licensing information see page 121.

Remarks on protein expression

Formation of disulfide bonds

Some vectors provide a N-terminal fusion of the **ompA signal peptide** which mediates the secretion of the recombinant protein to the periplasmic space of *E. coli*. There, the signal peptide is selectively cleaved by the *E. coli* signal peptidase. The secretion strategy is essential for the functional production of **proteins containing structural disulfide bonds** that are often present in naturally secreted proteins. The reducing conditions in the cytoplasm of *E. coli* prevent disulfide bond formation which can lead to aggregation or degradation of unfolded polypeptides.

Periplasmic secretion as a first purification step

Furthermore, periplasmic secretion separates the recombinant protein from cytosolic proteases. Since the *E. coli* outer membrane can be selectively degraded by mild treatment (EDTA, lysozyme etc.) the spheroplasts containing the cytosolic components can be easily removed by centrifugation.

Addition of active substances

In addition, the periplasmic space is accessible to molecules < 600 Da allowing to **influence folding or stability** of the recombinant protein during expression by adding active substances to the culture media (e.g. redox components, non-metabolizable sugars, ligands of the recombinant protein etc.).

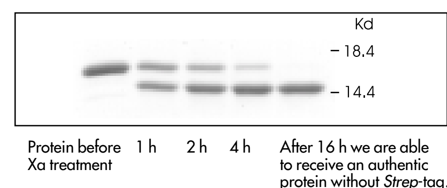
Cytoplasmic or periplasmic expression

As long as a cytoplasmic recombinant protein does not include stop-transfer sequences, the advantages of periplasmic secretion are also amenable to this type of protein. However, because stop-transfer sequences are difficult to predict, it is advisable to **try both strategies in parallel**. Using the vectors for N-terminal *Strep*-tag II or 6xHis-tag fusion, the change from cytoplasmic to periplasmic expression (and vice versa) can be achieved by a simple cloning step via the *Nhe*I/*Bst*BI and the *Eco*RV/HindIII restriction sites on the 5'- and 3'- end, respectively (see Appendix).

Do you need an authentic protein?

For special applications requiring an authentic protein, several vectors encoding the **factor Xa protease cleavage site** adjacent to *Strep*-tag II/6xHis-tag are available allowing the complete removal of the tag. In addition to factor Xa protease cleavage sites, we offer vectors with thrombin and enterokinase cleavage sites. For an overview of all vectors refer to page 44. Please note, that in most cases it is **superfluous to remove the tag**.

Factor Xa processing



Protein before Xa treatment **1 h** **2 h** **4 h** **After 16 h we are able to receive an authentic protein without *Strep*-tag.**

Protein gel demonstrating the treatment of the 15 kDa selenoprotein (carrying a N-terminal *Strep*-tag) with factor Xa (1:1000, w/w) at 22°C. The gene encoding the protein had been cloned into pASK-IBA6, which contains a Xa cleavage site adjacent to *Strep*-tag II (see polylinker in the Appendix).

Lane 1, protein before Xa processing; lane 2, after 1 hour incubation; lane 3, after 2 hours; lane 4, after 4 hours; lane 5, after 16 hours: homogenous authentic protein without *Strep*-tag II.

Cloning system and Primer D'Signer

Cloning procedure

The polylinkers of the expression vectors carry the restriction sites *Bsa*I (isoschizomer *Eco*31I) and *Bsm*FI (New England Biolabs, MBI Fermentas) which allow **the precise fusion of the structural gene with the vector-encoded functional elements** (including *Strep*-tag® II, 6xHis-tag and, depending on the vector, *OmpA*-signal sequence, start codon, protease cleavage site or stop codon, see pages 43-46). This is easily achieved by adapting both ends of the coding region of the structural gene via PCR.

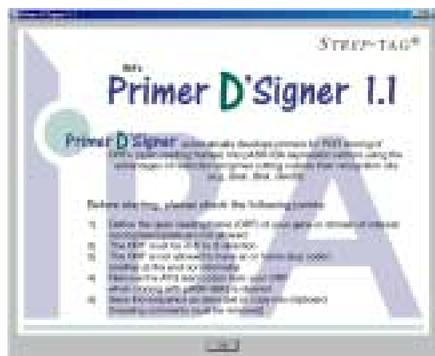
The cloning strategy is described for pASK-IBA3 (see page 49). If a different vector is to be used, the cloning strategy has to be adapted accordingly. The essential primer sequences for each vector are described in the Appendix or may be deduced by using the Primer D'Signer software (see below).

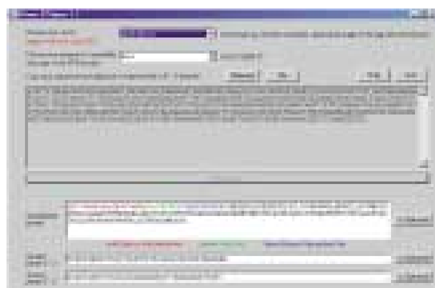
In cases where other restriction sites are intended to be used for cloning, care must be taken to ensure the in-frame fusion of the structural gene and the vector encoded functional elements.

In the vectors pASK-IBA4 to pASK-IBA7, pASK-IBA35, pASK-IBA37, pASK-IBA44 and pASK-IBA45 with N-terminal affinity tags (see page 44) the tag is followed by the **linker sequence** 5'-GGCGCC-3', which is recognized by four different restriction enzymes (*Kas*I, *Nar*I, *Ehe*I and *Bbe*I). These four enzymes cut the linker sequence in four different ways. Thus, cleavage with the suitable enzyme and a subsequent filling reaction enable the production of blunt ends in all reading frames in case the target gene insert requires a particular reading frame.

For phosphorothioate-protected primers see page 83.

To avoid the incorporation of base substitutions, PCR should be performed with a proof-reading DNA polymerase such as *Pfu* (Stratagene). 3' phosphorothioate-protected primers should be used in order to avoid 3'→5' degradation by the proof-reading activity (see page 83).





Free Primer D'Signer 1.1 Software for pASK-IBA and pPR-IBA Vectors

To facilitate the design of primers for PCR cloning of ORFs (open reading frames) into pASK-IBA and pPR-IBA expression vectors we now offer the new version of Primer D'Signer (1.1). The Windows software is available **free of charge for download on our web site www.iba-go.com**. Version 1.1 is suitable for all new pASK-IBA and pPR-IBA vectors (see page 44 and www.iba-go.com).

The **easy-to-handle software reduces boring primer design work to several mouse clicks only**. It checks the reading frame, looks for gene internal restriction sites and provides the optimal cloning strategy. Therefore, Primer D'Signer 1.1 is extremely convenient for all *Strep*-tag/6xHis-tag expression system users interested in e.g. the expression of authentic proteins.

Cloning scheme (demonstrated for pASK-IBA3)*

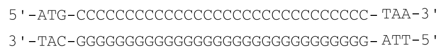
Precise fusion using *Eco*31I or *Bsa*I

Identification of start and stop codon of the target gene

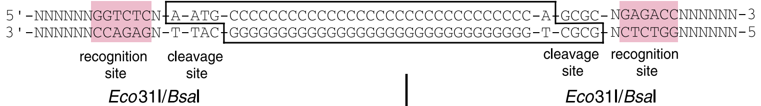
Primer construction

Forward primer

Reverse primer



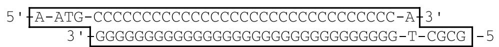
PCR Product



recognition site · cleavage site · *Eco*31I/*Bsa*I

cleavage site · recognition site · *Eco*31I/*Bsa*I

Digest with *Eco*31I or *Bsa*I



Ligation with pASK-IBA3
(digested with *Eco*31I or *Bsa*I)

ribosome binding site · linker 2 amino acids · *Strep*-tag 8 amino acids · stop codon

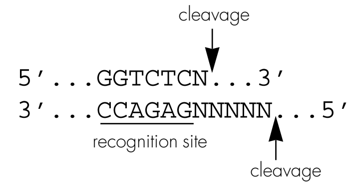
Construct
target gene + *Strep*-tag

ribosome binding site · start codon · target gene · linker 2 amino acids · *Strep*-tag 8 amino acids · stop codon

*Eco*31I and *Bsa*I belong to the Type IIS restriction enzymes which cleave the DNA double strand outside their recognition site (see below). Thereby, the digestion with one single enzyme can generate two different independent sticky ends with 4-base 5'-overhangs allowing directional cloning.

In addition, the digestion reaction removes the recognition sequence not affecting the encoded amino acid sequence and expressing authentic protein.

Eco31I/BsaI
Type IIS enzymes



* If a different vector is to be used, the cloning strategy has to be adapted accordingly. The essential primer sequences for each vector are described in the Appendix.

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Details see pages 68-71.

Tools for High-throughput protein expression and purification

Strep-Well HT Purification Plates

The perfect solution for automated, high-throughput purification of *Strep*-tag® proteins

New

Strep-Well HT Purification Plates

The perfect solution for automated, high-throughput purification of *Strep*-tag® proteins

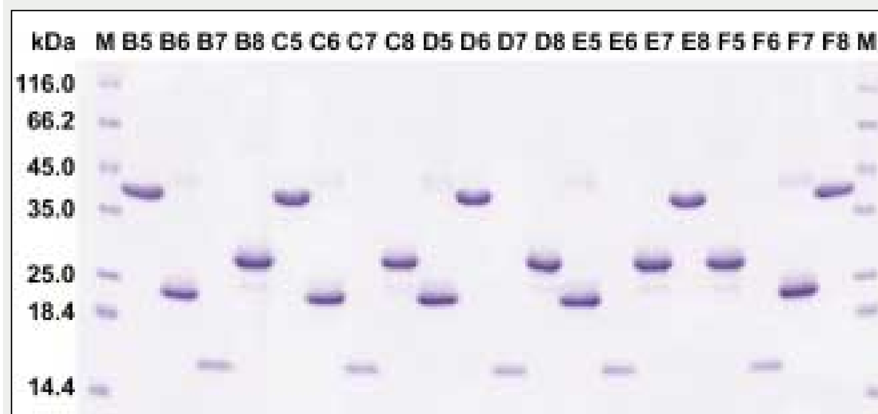
Features and benefits

- Simultaneous purification of up to 96 different *Strep*-tag proteins
- Up to 200 µg highly pure *Strep*-tag protein per well
- Convenient ready-to-use format
- Pre-filled with immobilized *Strep*-Tactin®
- Time-saving
- Compatible with vacuum manifolds and robotic systems

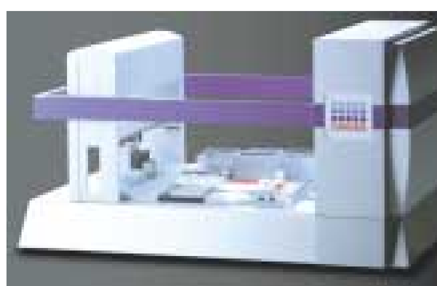
96 protein samples purified in parallel

Two different *Strep*-Well HT Purification plates are available: *Strep*-Well HT 25 Purification Plates provide automated protein purification of up to 100 µg recombinant protein per well, while *Strep*-Well HT 50 Purification Plates provide purification of up to 200 µg recombinant protein per well of 96 samples in parallel. These 96-well affinity chromatography modules are the perfect solution for **rapid or high-throughput purification of *Strep*-tag proteins.** The plates are pre-loaded with *Strep*-Tactin and simply have to be re-hydrated and equilibrated before usage.

Genesis RSP/RWS Separation System (Photo kindly provided by Tecan).



Strep-tag vector constructs for the expression of GAPDH from *S. aureus* (37.5 kDa), GFP from *A. victoria* (28.1 kDa), human GSHH (23.5 kDa) and Azurin from *P. aeruginosa* (15.1 kDa) were transformed into *E. coli* and plated on selective medium. 96 colonies were randomly picked to inoculate 5 ml cultures. Protein expression was induced by addition of anhydrotetracycline or IPTG. Cells were harvested, lysed and *Strep*-tag proteins were purified on a *Strep*-Tactin HT Purification Plate. 8 µl of 20 elution fractions were loaded on a SDS-PAGE, gel was stained with Coomassie.



BioRobot workstation (Photo kindly provided by Qiagen).

Compatibility with robotic systems

The ***Strep*-Well HT Purification Plate** is compatible with robotic sample processing systems, such as the Genesis RSP/RWS Separation System (Tecan), the MultiPROBE II (Packard BioScience), the BioRobot workstations (Qiagen) and the Biomek 2000/FX robots (Beckman Coulter), but can also be used with standard vacuum manifolds for manual sample processing.

Biomek FX robot (Photo kindly provided by Beckman Coulter).

For prices see separate price list or www.iba-go.com •info@iba-go.com

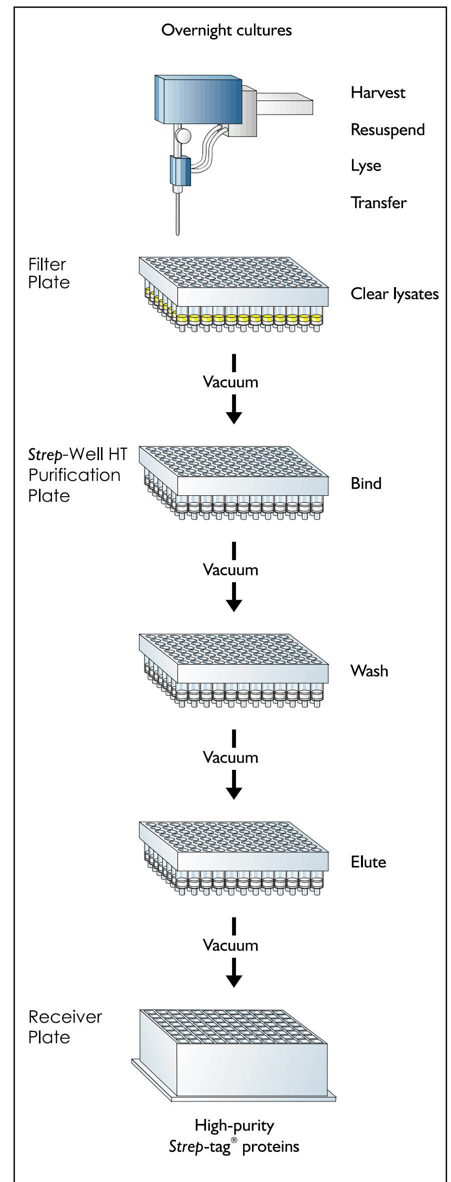
Procedure

The procedure uses a 96-well filter plate to clear bacterial lysates by vacuum filtration.

1. The cultures are chemically lysed.
2. The lysates are cleared by vacuum filtration through the **96-well filter plate.**
3. The cleared protein lysates are applied to the ***Strep*-Well HT Purification Plate**.
4. After washing, the purified proteins are eluted in 300-450 µl elution buffer.

To prevent cross-contamination of the samples a **96-well wash plate** is available. The eluates are collected in a **96-well receiver plate**.

Washing and elution steps are carried out on a vacuum manifold. Ready-to-run protocols are provided.



Protein purification using the Strep-Well HT Purification System

Strep-Well HT Purification Starter Kits

For a first evaluation we are offering a *Strep*-Well HT Purification Starter Kit in two versions, one with a *Strep*-Well HT 25 Purification Plate allowing purification of up to 100 µg protein per well and one with a *Strep*-Well HT 50 Purification Plate for the purification of up to 200 µg protein per well. Also included in the kits are one 96-well filter, wash and receiver plate each as well as the required buffers for processing 96 samples.

Larger amounts of plates and buffers are available separately (see below).

Strep-Well HT Purification Starter Kit

product	amount	cat. no.
Strep-Well HT 25 Purification Starter Kit	1 kit	2-1700-000
Strep-Well HT 50 Purification Starter Kit	1 kit	2-1701-000
Strep-Well HT 25 Purification Plates	10 plates	2-1725-010
Strep-Well HT 25 Purification Plates	25 plates	2-1725-025
Strep-Well HT 25 Purification Plates	100 plates	2-1725-100
Strep-Well HT 50 Purification Plates	10 plates	2-1750-010
Strep-Well HT 50 Purification Plates	25 plates	2-1750-025
Strep-Well HT 50 Purification Plates	100 plates	2-1750-100
Strep-Well HT buffer set for 10 plates		2-1702-000
Strep-Well HT buffer set for 25 plates		2-1703-000
Strep-Well HT buffer set for 100 plates		2-1704-000
Strep-Well HT filter plates	10 plates	2-1705-010
Strep-Well HT wash plates	10 plates	2-1706-010
Strep-Well HT receiver plates	10 plates	2-1707-010

Further *Strep*-Well HT Purification Plate formats on request. See also our custom service on page 57 ff.
Strep-tag vectors are described on pages 41 ff.