iba Solutions For Life Sciences

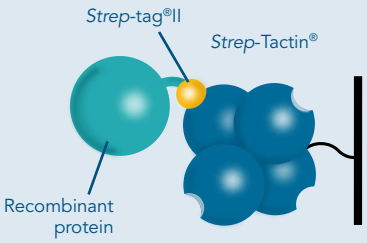
The 3rd generation

STREP-TACTIN® XT AND TWIN-STREP-TAG®

Unparalleled Performance!

THE *STREP*-TAG®- TECHNOLOGY

2nd generation



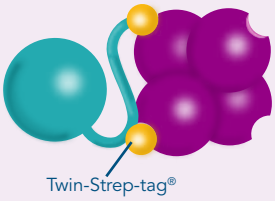
1. *Strep*-tag®II

- Only 8 aa with a neutral aa composition
- Rapid one-step purification under physiological conditions
- No tag removal necessary

2. *Strep*-Tactin®

- Purification of *Strep*-tag®II and Twin-Strep-tag® proteins
- Detection of *Strep*-tag®II and Twin-Strep-tag® proteins
- High purity due to low tendency of *Strep*-Tactin® for unspecific protein binding

3rd generation

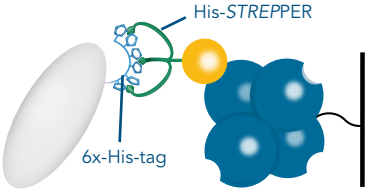


3. Twin-Strep-tag®

- Higher affinity for *Strep*-Tactin®, same mild elution conditions
- More efficient capture of proteins from diluted cell culture supernatants

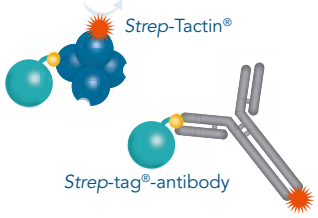
4. *NEW* Strep-Tactin® XT (xtra tight)

- New developed variant for immobilization/assays
- Allows purification under denaturing conditions
- Affinity with Twin-Strep-tag® in pM range



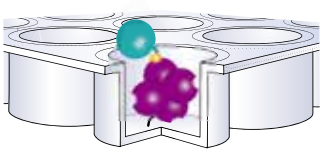
5. His-*STREP*PER

- Adapter molecule which alters His-tag fusion proteins into *Strep*-tag® fusion proteins
- No cloning required



6. Detection and Visualization

- *Strep*-Tactin® and *Strep*-tag® antibodies for detection
- Fluorescent conjugates
- HRP and AP conjugates



7. Immobilization

- Twin-Strep-tag® binds to Strep-Tactin® XT in pM range
- Very low off-rate but mild elution still possible

CONTENT

Why we use *Strep*-tag®!

"Why we admire the Strep®-tag purification system: (i) generally good expression of recombinant proteins, (ii) simplicity of protein purification and very high purity of isolated proteins (the latter is very important for crystallographic projects!), (iii) robust system to generate, reliably purify, and biochemically analyze various mutant derivatives reproducibly."

Prof. Dr. Erhard Bremer, Laboratory for Microbiology, Department of Biology, Philipps University Marburg, Germany.

"The Strep II tag appears to be an excellent candidate for affinity purification in general since it is a short tag that produces high purity material in good yields at a moderate cost. / ... / we find that a combination of His-tag and StrepII tag allows rapid capture of the tagged protein or protein complex from crude extracts..."

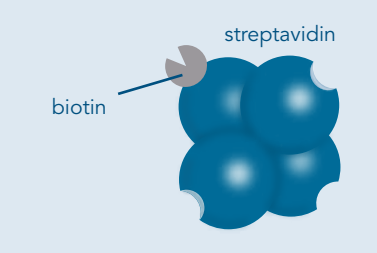
Lichty *et al.* 2005, Protein Expression and Purification 41: 98-105.

1. TECHNOLOGIES

1.1 *STREP*-TAG®II

We offer a wide range of high quality *Strep*-tag®II products, such as

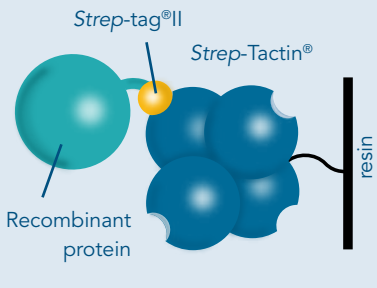
- **Cloning/Expression Vectors** for different hosts
- Different *Strep*-Tactin® purification **resins** and pre-packed **columns**
- **Detection** systems with *Strep*-Tactin® conjugates or a monoclonal **antibody**
- Products for **immobilization** of fusion proteins



The *Strep*-tag®II principle is based on the specific interaction of biotin with streptavidin

***Strep*-tag®II – the short 8-amino acid tag does not interference with the protein.**

NH_2-SA-**WSHPQFEK**-COOH



Recombinant protein with *Strep*-tag®II bound to *Strep*-Tactin®.

Benefits of *Strep*-tag®II

- Highly pure proteins
- Fast and easy purification procedure
- Flexible buffer and purification conditions
- Regeneration of resins for re-use

Introduction

The *Strep*-tag®II is a short peptide consisting of 8 amino acids. It was initially developed for the specific reversible binding to the biotin pocket of streptavidin.
Streptavidin was later on engineered to obtain the highly selective *Strep*-Tactin®, which binds the *Strep*-tag®II with a nearly 100 times higher affinity compared to streptavidin.
The *Strep*-tag®II can be fused to the protein as either N- or C-terminal tag. Physiological buffers like PBS in combination with a wide range of additives can be used. The competitive elution is performed with desthiobiotin, an inexpensive, reversibly binding and stable analog of biotin.

Applications

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

- Structural and functional investigations
- Crystallization for determination of 3D structure
- Assays involving protein-protein and protein-DNA interactions
- Investigating ligand-receptor interactions under physiological conditions
- Separating living cells for re-culturing purposes

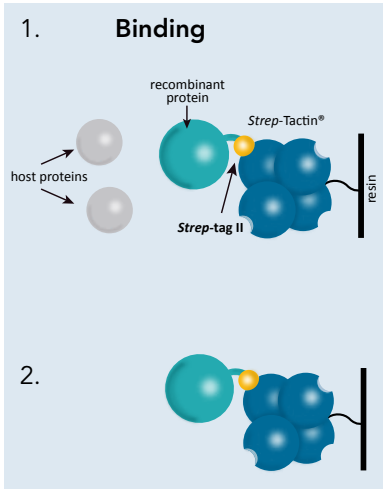
Strep-tag®II is the method of choice for multiple protein classes:

- Metalloproteins
- Membrane proteins
- Fragile protein complexes with multiple subunits
- And any other protein

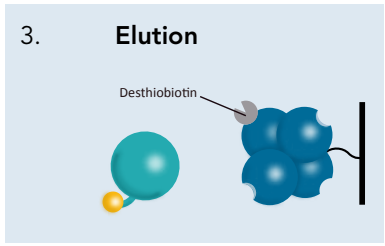
Workflow of *Strep*-tag®II and Twin-Strep-tag®

Physiological purification conditions preserve the structure of the protein and its functionality

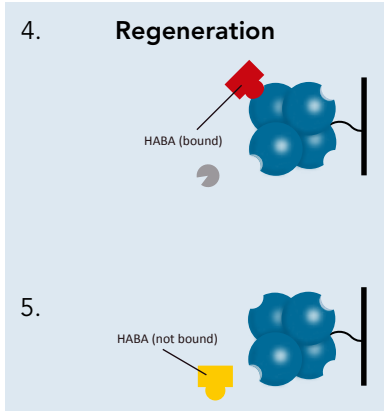
The purification of *Strep*-tag®II fusion proteins is **easy, straightforward and user-friendly.** The complete procedure should be performed under physiological-like conditions, e.g. in PBS buffer pH >7.0.



Steps 1 + 2: The cell lysate is subjected to the column. Once the tagged protein of interest has **bound specifically to *Strep*-Tactin®** the host proteins are rapidly washed off due to addition of small amounts of physiological wash buffer.



Step 3: In a next step, bound *Strep*-tag®II protein is **gently eluted** by addition of wash buffer supplemented with 2.5 mM desthiobiotin, which specifically competes for the biotin binding pocket. Since the **buffer conditions** during elution essentially **remain unchanged** (ionic strength, pH), unspecific bound proteins (without *Strep*-tag®II) will not co-elute and, thus, will not contaminate the protein of interest. Besides the specific binding of *Strep*-tag®II to *Strep*-Tactin®, this is the second specificity conferring step of the purification procedure, thereby yielding extremely high protein purity.



Steps 4: In order to regenerate the column, the yellow azo dye HABA (2- [4'-hydroxy-benzeneazo] benzoic acid) is added in excess to displace desthiobiotin from the binding pocket. Once HABA binds to the binding site, the color of columns turns to red. This **indicates the regeneration process and activity status** of the column.

Step 5: HABA can be removed simply by rinsing with wash buffer. Once the red color has disappeared, the column can be re-used.

Note:

- Crucial for proper binding of the tagged proteins to *Strep*-Tactin® is a pH>7.0 (better pH 8.0) during the purification cycle (if this is not possible see page 18)
- Use biotin for elution of *Strep*-tag®II or Twin-Strep-tag® fusion proteins from Strep-Tactin® XT resin
- Regeneration of the columns is only possible after elution with desthiobiotin (not after biotin elution)

Strep-Tactin® columns regenerated with HABA

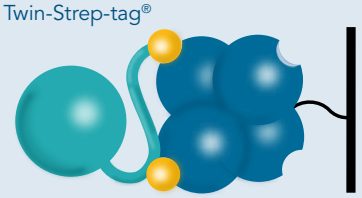
1.2 TWIN-STREP-TAG®

Twin-Strep-tag® the high affinity *Strep*-tag®II

- Purification of highly diluted proteins, e.g. from cell culture supernatants
- For proteins where *Strep*-tag®II does not work
- Large proteins, e.g. Protein complex isolation (see page 7)
- Membrane proteins - additives and detergents required
- pM affinity for Strep-Tactin® XT - stable binding in assays (see page 8)

Twin-Strep-tag® is the tag of choice for **highly diluted target proteins**, e.g. from cell culture supernatants.

Twin-Strep-tag®



Twin-Strep-tag®: tandem arrangement of two *Strep*-tag®II (total size of 30 aa) results in higher affinity

SA-WSHPQFEK(GGGS)$_2$GG**SAWSHPQFEK**

The tandem arrangement of two *Strep*-tag®II, in the Twin-Strep-tag® avoids **influences of the protein of interest on its binding to *Strep*-Tactin®**.

Twin-Strep-tag® is a sequential arrangement of two *Strep*-tag®II sequences. This tag enables the same mild and rapid purification as *Strep*-tag®II. It has a significantly increased affinity for *Strep*-Tactin® and an improved binding because of its lager size. Negative effects of the fusion protein on the short *Strep*-tag®II are efficiently reduced.

Benefits of the Twin-Strep-tag®

- Neutral amino acid composition
- High affinity leads to higher yields of protein
- Tolerates elevated levels of additives and detergents

Fusion protein	$T_{1/2}$	K_D [nM]	Affinity increase
GFP-StrepII	23.0 s	300	
GFP-Twin-Strep	161.0 s	57	**x 5**
Cytb$_{562}$-StrepII	2.4 s	16,700	
Cytb$_{562}$-Twin-Strep	120.0 s	97	**x 150**

@ < 500 RU *Strep*-Tactin®

Table: The Twin-Strep-tag® increases binding affinity with *Strep*-Tactin® significantly
Comparison of two model proteins fused with *Strep*-tag®II with good (GFP) and poor (Cytb562) binding affinities to *Strep*-Tactin®.

Deeper insights into the *Strep*-tag® technology can be found here:

Schmidt & Skerra (2015) **"The Strep-tag system for one-step affinity purification of proteins from mammalian cell culture"** Methods Mol Biol. 1286: 83-95, doi: 10.1007/978-1-4939-2447-9_8

Schmidt et al. (2013) **"Development of the Twin-Strep-tag® and its application for purification of recombinant proteins from cell culture supernatants"** Protein Expression and Purification, volume 92, Issue 1

Schmidt & Skerra (2007) **"The Strep-tag system for one-step purification and high affinity detection or capturing of proteins"** Nat Prot 2(6): 1528-35

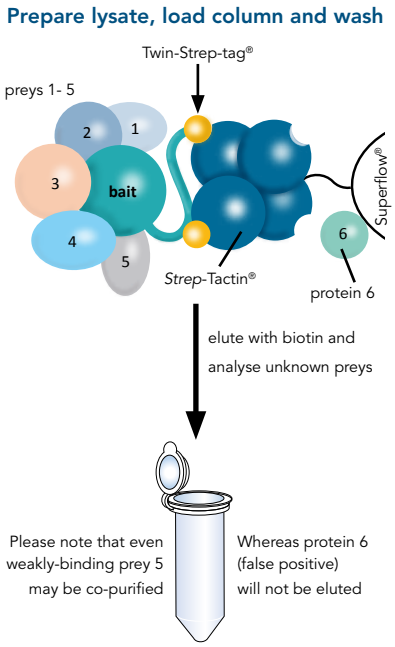
Protein:protein interaction analysis

The high specificity of the Twin-Strep-tag®:*Strep*-Tactin® interaction and low tendency of *Strep*-Tactin® to bind proteins non-specifically makes the system a widely used and outstanding tool for the identification of protein interaction partners.

Twin-Strep-tag® is especially used in purification of **protein complexes**, given that its size has nearly no influence on the complex formation *in vivo* compared to protein tags, like MBP or GST.

Features:

- Isolation of interaction partners after one purification step
- Better availibility of the tag due to the sequential arrangement of two *Strep*-tag®II
- High specificity – low tendency to isolate false positive protein preys
- Tolerates elevated levels of additives and detergents
- Mild, physiological purification conditions preserve the structure of protein complexes

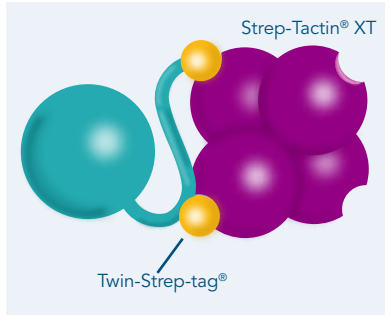


References:

1. Correa & Oppezzo, 2015, Methods Mol Biol. 1258: 27-44
2. Lin *et al.*, 2015, Biochem Biophys Acta. 1854(3): 198-208
3. Ivanov *et al.*, 2014, J Vis Exp. 20 (86)
4. Johansen *et al.*, 2008, J Cell Sci 121: 854-864
5. Groth *et al.*, 2007, Science 318: 1928-1931

Twin-Strep-tag and Strep-Tactin® XT – two for stable immobilization and assay implementation

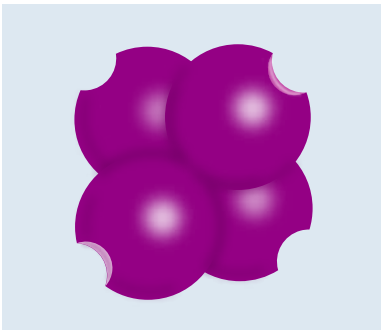
The high affinity of Twin-Strep-tag® to the recently developed Strep-Tactin® XT (see next chapter) improves the performance of the *Strep*-tag® technology in the field of assay applications due to the high binding affinity and washing stability.



Features:

- pM affinity of Twin-Strep-tag® to Strep-Tactin® XT – very stable binding
- Reversibility of Twin-Strep-tag®:Strep-Tactin® XT binding
- High specificity – low background
- Stable under physiological conditions
- Allows usage of detergents and additives

1.3 STREP-TACTIN® XT



Strep-Tactin® XT

X-tra **T**ight binding of *Strep*-tag® II and Twin-Strep-tag® for assays

In order to improve the *Strep*-tag® performance for assay applications Strep-Tactin® XT was developed.
Strep-Tactin® XT has a binding affinity in low pM ranges for Twin-Strep-tag® and in nM range for *Strep*-tag®II. This improvement makes the *Strep*-tag® technology suitable for new assay applications.

Benefits of Strep-Tactin® XT:

- Allows the immobilization in very stable ranges ($T_{1/2}$ = 13 h)
- Improves the performance of the *Strep*-tag® technology in assays
- Purification even under denaturing conditions possible
- Binding is still reversible for mild recovery of immobilized proteins

The leading technology for protein purification:

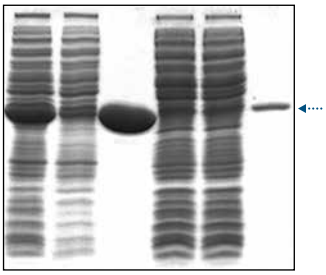
10 reasons to use Strep-tag!

1. **Highly pure** proteins (> 95 %)
2. **Functional** proteins due to physiological conditions
3. **Low non-specific binding** in a fast one-step purification requiring a low washing volume
4. **Variable buffer** conditions; high salts, detergents, metal
5. **Low background** – specific *Strep*-tag®:*Strep*-Tactin® interaction and competitive elution with desthiobiotin
6. **Economic: re-usable**, robust purification resins
7. **Preserves protein complex integrity** – mild elution conditions, low washing volumes
8. **Efficient immobilization** via Strep-Tactin® XT (reversible binding) or *Strep*MAB-Immo (non-reversible)
9. **No tag removal** required due to neutral pI. It does not influence protein folding and function
10. **Universal** detection system for Western blot, ELISA, Immunofluorescence, FACS and more …

(TWIN-) *STREP*-TAG®:STREP-TACTIN® APPLICATION EXAMPLES

Pure proteins independent of expression host

Bacterial
(*E.coli*)

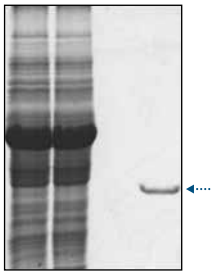


Mannitol-1-phosphatase
Eimeria tenella

Yeast
(*S.cerevisiae*)

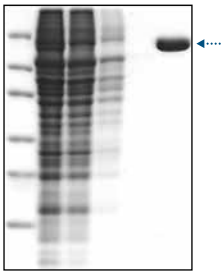
Phytochrome A
Avena sativa

Plant
(*Nicotiana benthamiana*)



Ureidoglycine
aminohydrolase
Arabidopsis thaliana

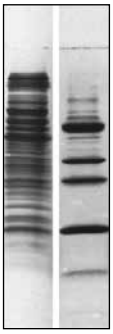
Mammalian
(HEK293)



Tissue transglutaminase
human

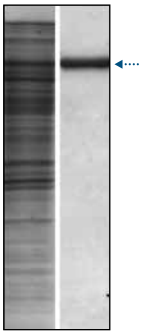
Pure proteins independent of protein class

Multimeric membrane
protein complexes



Cytochrome c
oxidase

Large proteins

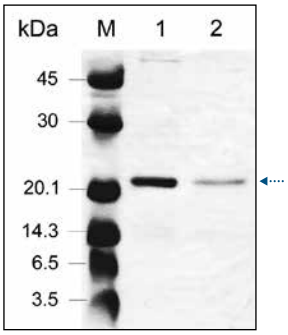


Oat phytochrome A
124kDa

Metalloenzymes

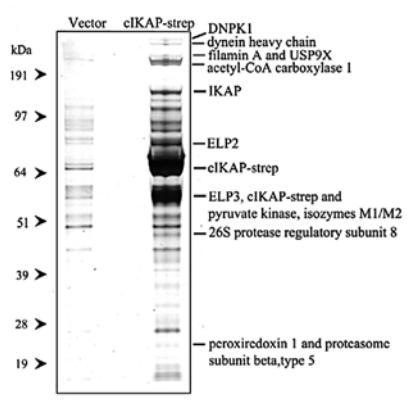
Alkaline
Phosphatase

VLPs
(4.8MDa: virus like particle)

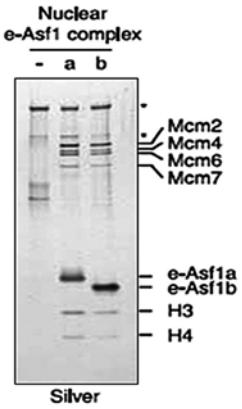


4.8 mDa capsides consist of
240 x 20 kDa monomers

Co-purified associated proteins in protein:protein interaction analysis

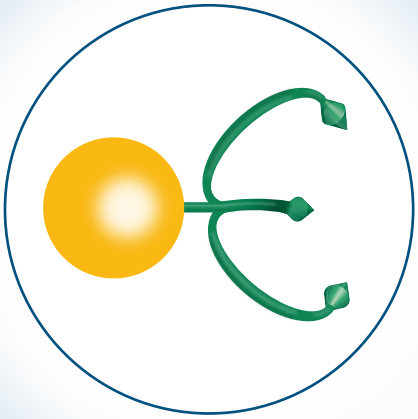


cIKAP-Twin-Strep - 15 associated
proteins were identified

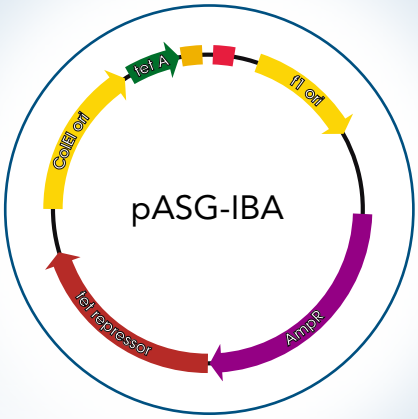


Twin-Strep-tagged histone chaperones Asf1a or
Asf1b - 6 associated proteins were identified

Strep-tag® Product Portfolio

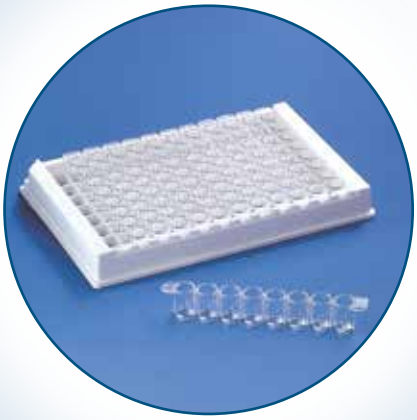


His-*STREP*PER

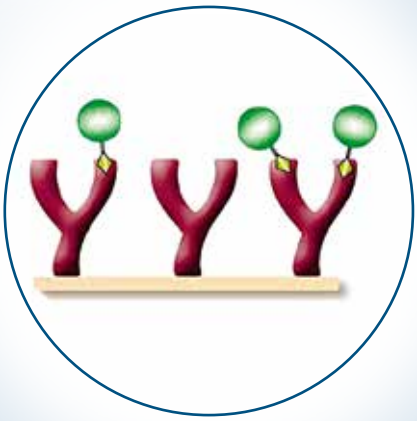


Expression

Purification



Immobilization

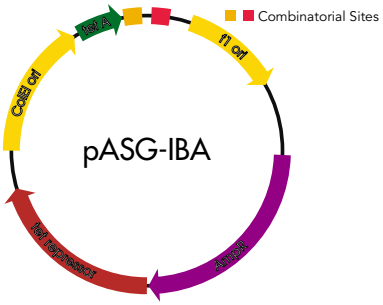


Detection

2. PRODUCTS

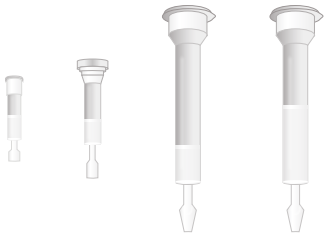
2.1 EXPRESSION VECTORS

- Expression vectors
  - *E. coli*, mammalia, insect cells and yeast
  - *Strep*-tag®II, Twin-Strep-tag®, His-tag, GST-tag, FLAG-tag
  - Protease cleavage sites (Factor Xa, TEV, PreScission, Thrombin; Enterokinase)
- MEXi - Optimized Mammalian Expression System



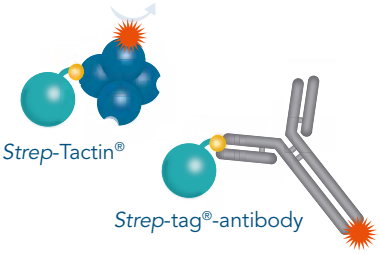
2.2 PURIFICATION RESINS AND REAGENTS...

- Starter Kits for *Strep*-tag®II and Twin-Strep-tag®
- WET FRED: application tool for large culture volumes
- Buffers and Reagents
- Different *Strep*-Tactin® resins
- Mag*Strep* – *Strep*-Tactin® coated magnetic beads
- Columns, cartridges, plate formats and adapters



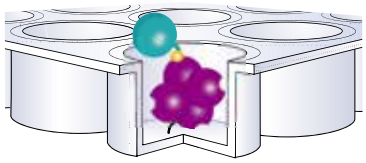
2.3 DETECTION REAGENTS

- Two different detection systems are available:
  - Monoclonal antibodies, conjugated or unconjugated
  - *Strep*-Tactin® conjugates
- Applications: Western Blot, ELISA, FACS and Immunofluorescence



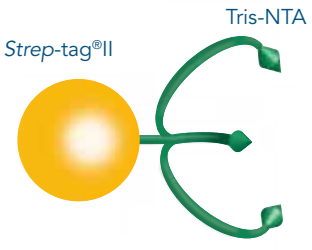
2.4 IMMOBILIZATION PLATES

- Two different detection systems are available:
  - Via Strep-Tactin® XT
  - Via StrepMAB Immo
- Applications: ELISA, antibody or serum screening, diagnostic assays, protein interaction studies, screening of engineered enzymes, …

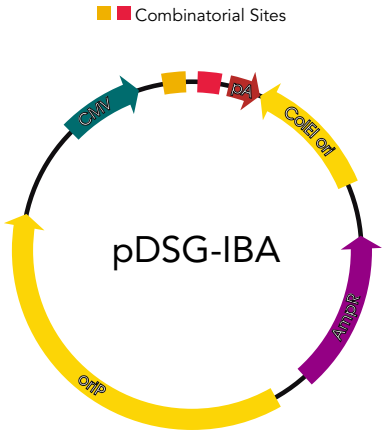


2.5 His-tag POLISHING PRODUCTS

- His-*STREP*PER - Clean-up your His-tag protein
- Double-tag purification of highly pure full-length protein



His-*STREP*PER



2.1 EXPRESSION VECTORS

IBA offers 187 different expression vectors

- For *E. coli*, mammalia, insect cells and yeast
- *Strep*-tag®II, Twin-Strep-tag®, 6xHis-tag, GST-tag and FLAG-tag
- Protease cleavage sites (Factor Xa, TEV, Enterokinase etc.)
- Ampicillin and Chloramphenicol resistance for *E. coli*

Visit our homepage for the complete vector portfolio:
www.iba-lifesciences.com/strep-tag-expression-vectors-technology.html

Available features of expression vectors:

Host	Vector series	Promoter	Secretion (optional)	Protease cleavage sites	Available tags	Resistance
E. coli	pASK-IBA	Tet	OmpA	Factor Xa, TEV, Enterokinase, Thrombin	*Strep*-tag®II Twin-Strep-tag® His-tag GST-tag FLAG-tag®	Amp, CAT
	pASG-IBA					Amp
	pPSG-IBA	T7				
Mammalia	pEXPR-IBA	CMV	BM40	Factor Xa, TEV, Enterokinase, Thrombin		
	pDSG-IBA*					
	pESG-IBA					
	pCSG-IBA*					
Insect cells	pLSG-IBA	Polyhedrin				
Yeast	pYSG-IBA	CUP1				

* Contain oriP/EBNA-1 for episomal expression

MEXi – IBA's Mammalian Expression System

The MEXi (**M**ammalian **Ex**pression **I**BA) system was developed to provide an optimized system for episomal expression of Twin-/*Strep*-tag®II fusion proteins in mammalian HEK293E suspension cells.
It consits of a HEK293E cell line (MEXi 293E), a transfection (MEXi®-TM) and culture (MEXi-CM) medium as well as an optimized vector system (pDSG-IBA).

! Use for:

Expression of (Twin-) *Strep*-tag® fusion proteins in an optimized mammalian expression system

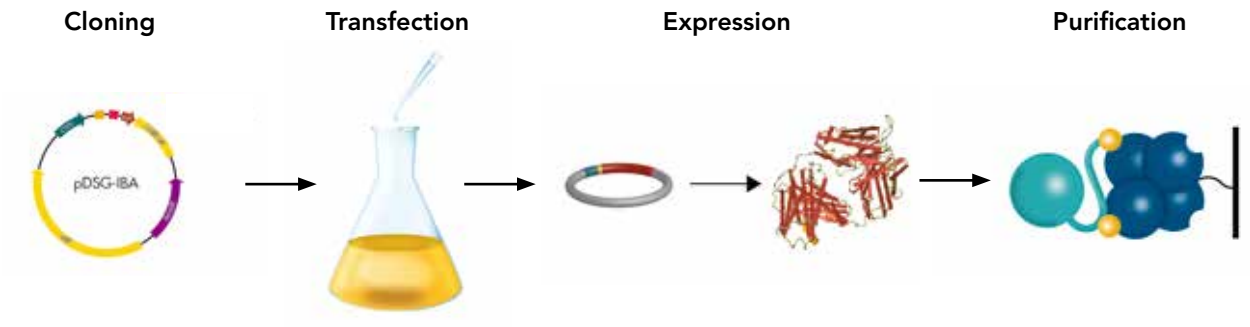
Features:

- High protein yields
- Easy handling
- Optimized components
- Transient expression for time and cost efficient protein production

MEXi 293E cell line	Cat.no. 2-6001-001
MEXi Culture Medium (MEXi-CM)	Cat.no. 2-6010-010
MEXi Transfection Medium (MEXi-TM)	Cat.no. 2-6011-010
pDSG vector system	

(see www.iba-lifesciences.com/expression-vector-overview.html)

Workflow:



References:

Schmidt & Skerra (2015) **"The Strep-tag system for one-step affinity purification of proteins from mammalian cell culture"** Methods Mol Biol. 1286: 83-95, doi: 10.1007/978-1-4939-2447-9_8

Schmidt et al. (2013) **"Development of the Twin-Strep-tag® and its application for purification of recombinant proteins from cell culture supernatants"** Protein Expression and Purification, volume 92, Issue 1

2.2 PURIFICATION RESINS AND REAGENTS

Strep-tag® Starter Kit

Strep-tag®II Starter Kits

Our *Strep*-tag®II Starter Kits are an attractive offer for newcomers and experts. They contain all essential reagents required for expression in *E. coli*, purification and detection of *Strep*-tag®II proteins.

Two Starter Kits are available :

Strep-tag® Starter Kit
Contains one ready-to-use gravity flow column with *Strep*-Tactin® Sepharose®.
Cat. no. 2-1101-000

Strep-tag® Starter Kit 3C
Includes 3 different gravity flow columns with *Strep*-Tactin® immobilized to Sepharose®, MacroPrep® and Superflow®, respectively, allowing the evaluation of the optimal resin for your particular protein of interest (more information about resin can be found on page 16/17).
Cat. no. 2-1102-001

Both *Strep*-tag®II Starter Kits include:

- Control plasmid with a 15 kD protein insert
- Anhydrotetracycline for induction of expression
- Fractionation buffer for the preparation of a periplasmic extract
- Wash buffer for column chromatography and for the preparation of a cytoplasmic extract
- Elution buffer for displacing the *Strep*-tag®II protein from the column
- Column regeneration buffer (with HABA)
- *Strep*-Tactin® horse radish peroxidase (HRP) conjugate for Western blot detection

Twin-Strep-tag® - Starter Kit

Twin-Strep-tag® purification basically works with the same products as *Strep*-tag®II purification. Therefore, the same Starter Kits can be used.
Furthermore, Twin-Strep-tag® is recommended for protein:protein interaction (PPI) analysis. For the different described PPI analysis methods specific purification kits are available :

Twin-Strep Basic Purification Kit	Cat.no. 2-1121-010
Twin-Strep Purification Kit (*E. coli*/mammalia)	Cat.no. 2-1121-011/012
One-TAP Purification Kit	Cat.no. 2-1121-011/012
Two-TAP Purification Kit	Cat.no. 2-1121-011/012
SPINE Purifcation Kit	Cat.no. 2-1121-011/012

More information:
www.iba-lifesciences.com/IBA-Applications-Protein-interaction.html

Use for:

Twin-Strep-tag® is the tag of choice if size does not matter. Its higher affinity for *Strep*-Tactin® and especially Strep-Tactin® XT is advantagous particularly for challenging proteins and highly deluted proteins

Strep-tag®II:	8 aa
Twin-Strep-tag®:	30 aa

WET FRED

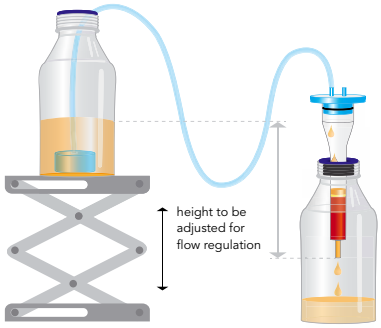
Applicator for purification of Twin-*Strep*-tag® fusion proteins from cell culture supernatants

Mammalian and insect cell expression systems are often used to secrete proteins into the cell culture medium. Thus, the protein is present in large volumes before the purification step. To apply this large volume onto a gravity flow column with continuous flow, the WET FRED applicator was developed. This device facilitates the transfer of large cell culture volumes to a *Strep*-Tactin® gravity flow column for purification of the recombinant target protein fused with (Twin-) *Strep*-tag®. It works by hydrostatic pressure (siphon principle). Due to its small size and flexibility, it is easy to handle at the bench, in the cold room or in the fridge. Columns cannot run dry and do not need supervision. Furthermore, no sophisticated software is necessary facilitating set up and use.

Cat.no. 2-0911-001 for 1ml columns and
Cat.no. 2-0910-001 for 5-10ml columns

Use for:

Applicator for large cell culture volumes



Experimental set-up

Which Resin to use?
Test our different *Strep*-Tactin® resins to evaluate the best one for the purification of your protein of interest. The ***Strep*-tag® Starter Kit 3C (2-1102-001)** consits of 1 ml gravity flow columns of *Strep*-Tactin®-Sepharose®, -Superflow® and -MacroPrep®, respectively.

Strep-Tactin® resins

Different types of *Strep*-Tactin® resins are provided
Several *Strep*-Tactin® resin versions are available which differ in their properties and suitability for applications:

Sepharose®	The resin **to start with** (if you have no requirements)! ⇢ Agarose support with good flow properties ⇢ For gravity flow chromatography only (not pressure stable) ⇢ Protein capacity: 50 – 100 nmol	
Superflow®	**Our all-rounder** for large proteins and protein:protein interaction analysis ⇢ Agarose support with low unspecific binding properties ⇢ For increased flow rates ⇢ Low pressure stable (FPLC/HPLC/Äkta applications) ⇢ Protein capacity: 50 – 100 nmol	
Superflow® high capacity (HC)	The capacity of Superflow® is not sufficient or the binding affinity to (Twin-)Strep-tag® fusion proteins needs to be slightly improved! ⇢ **3-5 fold higher binding capacity** as Superflow® ⇢ Protein capacity: 150 – 500 nmol	
MacroPrep®	The alternative if Sepharose® and Superflow® lead to non-specific binding of proteins (e.g. Chlorophyll from **plant extracts**) ⇢ Synthetic support with low non-specific binding properties ⇢ Highly pressure stable (FPLC/HPLC /Äkta applications) ⇢ Protein capacity: 150 – 500 nmol	
Mag*Strep* Beads (Strep-Tactin® XT coated magnetic beads)	For **small-scale purification** ⇢ For batch purification only. ⇢ Mag*Strep* "type3" XT beads allow efficient binding with low background (see page 18) ⇢ For 150 µg protein per column	
	Applied volumes	
	Application	
	Adaptors for Cartridges	

	 0.2 ml gravity flow columns (5 columns)	 1 ml gravity flow column	 5 ml gravity flow column	 10 ml gravity flow column	 1 ml cartrigde	 5 ml cartrigde	 Spin Columns
	2-1202-550	2-1202-001	2-1202-051	2-1202-101			
	2-1207-550	2-1207-001	2-1207-051	2-1207-101	2-1235-001	2-1236-001	
	2-1209-550	2-1209-001	2-1209-051	2-1209-101	2-1237-001	2-1238-001	
	2-1506-550	2-1506-001	2-1506-051	2-1506-101	2-1537-001	2-1538-001	2-1850-050 Kit: 2-1800-000 2-1850-010
							2-4090-002
	0.1 - 2 ml	0.5 - 10 ml	2.5 - 50 ml	5 - 100 ml	0.5 - 10 ml	2.5 - 50 ml	Up to 500 µl
	Gravity flow				FPLC/HPLC		Spinning
	Available on our homepage: www.iba-lifesciences.com						

MagStrep "type3" XT beads

Use for:

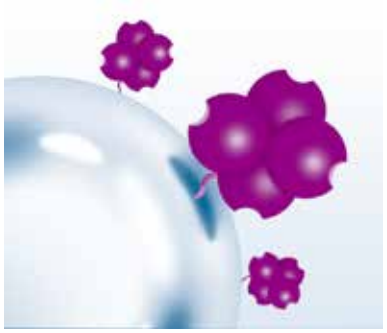
Small scale batch purification of Twin-*Strep*-tag® fusion proteins

- Superior Strep-Tactin® XT coat for highly efficient binding
- Magnetic beads for batch purification

MagStrep "type3" XT beads enable fast purification of Twin-*Strep*-tag® fusion proteins from small volumes in batch.

Features:

- High binding capacity (1-3 nmol/µl beads, corresponding to 30-90 µg of a 30 kDa protein)
- Very low non-specific protein binding due to improved coating
- Flexible elution conditions - under denaturing conditions by boiling in SDS gel loading buffer or under native conditions with biotin
- Improved binding for Twin-/*Strep*-tag®II – due to new Strep-Tactin® XT



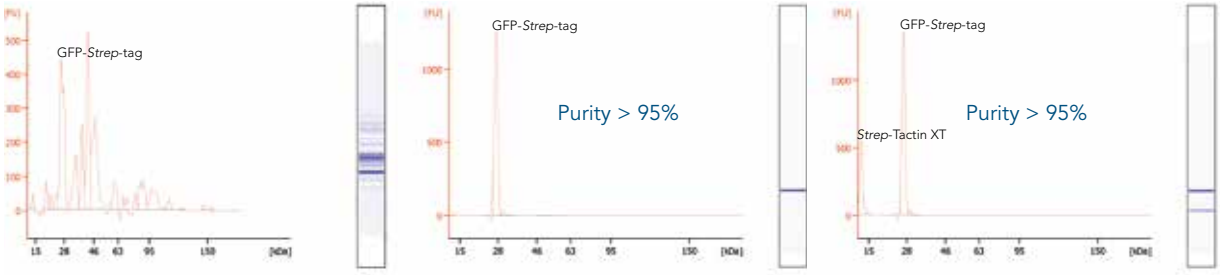
MagStrep "type3" XT bead

MagStrep "type3" XT beads:	Cat.no. 2-4090-002
Magnetic separator:	Cat.no. 2-1602-000
Biotin Elution Buffer (5x Buffer BX):	Cat.no. 2-1040-050

Crude bacterial extract before purification

Native purification using buffer BX for elution

Denaturing purification using sample buffer and boiling for elution



Purification of GFP-*Strep*-tag II fusion protein from crude bacterial extract using MagStrep "type3" XT beads. Due to specific binding properties of the beads even the elution by boiling leads to highly pure proteins. (Protein purification analysis was performed with an Agilent Bioanalyzer 2100 system)

Gravity flow StrepMAB-Classic MacroPrep® Column

Use for:

- Purification at pH < 7.0
- Tandem affinity purification

For purification at low pH and for TAP purification

This antibody-based purification column with *Strep*MAB-Classic antibody immobilized to MacroPrep® provides an alternative to the *Strep*-Tactin® based purification of *Strep*-tag®II proteins. The antibody based purification method might be useful for extracts where a pH lower 7.0 is required and for tandem affinity purification (TAP) in protein:protein interaction analysis. For TAP a combination of a *Strep*-Tactin® and *Strep*MAB-Classic based purification cycles are performed to reduce potential contaminations caused by unspecific bound proteins.

Cat.no. 2-1526-001 (1 ml); 2-1526-505 (5 x 0.2 ml)

Buffers and Reagents

Product	Contents	Cat.no
Anhydrotetracycline (inducer for tet promotor)	25 mg	2-0401-002
Avidin – for biotin blocking (high grade for PPI applications)	50 mg	2-0204-050
BioLock - biotin blocking solution (low grade)	50 ml	2-0205-050
Strep-tag® protein purification buffer set	100 ml 10x Buffer W 25 ml 10x Buffer E 100 ml 10x Buffer R	2-1002-001
D-Desthiobiotin (lyophilized)	1 g, 5 g	2-1000-002 2-1000-005
Elution Buffer with D-Desthiobiotin (10x Buffer E)	25 ml	2-1000-025
Strep-tag® regeneration buffer with HABA (10x Buffer R)	100 ml	2-1002-100
Strep-tag® washing buffer (10x Buffer W)	100 ml	2-1003-100
Strep-tag®II Peptide	1.8 mg	2-1018-002
Biotin Elution Buffer (10x Buffer BE)	25 ml	2-1019-025
Biotin Elution Buffer BX (5x Buffer BX) for MagStrep „type3" XT	50 ml	2-1040-050

BioLock biotin blocking solution

Mammalian and insect cell culture media often contain significant amounts of biotin. When proteins from biotin containing extracts or media are intended to be purified via *Strep*-Tactin® chromatography, biotin must be masked by the addition of avidin prior to the application onto the column. BioLock biotin blocking solution allows masking of biotin (and biotinylated proteins) in a very fast and convenient way.

Activity: >70 U/ml; add at least 1U of BioLock solution per µg of biotin.

Cat.no. 2-0205-050

Use for:

Masking of biotin from culture media (cost-effectice alternative to pure Avidin)

Biotin Elution Buffer BX

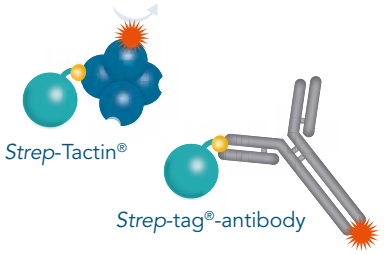
The new Strep-Tactin® XT variant requires a more competitive elution buffer due to the higher affinity of Strep-Tactin® XT for *Strep*-tag®II and especially Twin-Strep-tag®. Biotin Elution Buffer BX contains higher biotin concentrations (5x: 50 mM D-biotin) to enable efficient elution from e.g. Mag*Strep* "type3" XT beads.

Cat.no. 2-1040-050

Use for:

Elution from Strep-Tactin® XT

2.3 DETECTION REAGENTS



Two different detection systems are available to detect *Strep*-tag®II as well as Twin-Strep-tag® at the N-terminus or C-terminus:
a) Monoclonal antibodies (mAB) , conjugated and unconjugated
b) *Strep*-Tactin® conjugates

Following monoclonal antibodies against *Strep*-tag® are available:

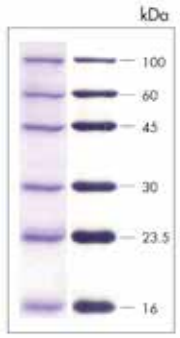
- ***Strep*MAB-Classic**, unconjugated
- ***Strep*MAB-Classic HRP conjugate** (HRP, horseradish peroxidase)
- ***Strep*MAB-Immo** - the high affinity antibody for capturing *Strep*-tag® proteins on solid surfaces

The *Strep*-tag® protein detection system supports a broad variety of assays including:

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

Product overview:

Detection System	a) Monoclonal antibodies		b) *Strep*-Tactin® conjugates	
	*Strep*MAB-Classic	*Strep*MAB-Classic HRP conjugate	*Strep*-Tactin® HRP conjugate	*Strep*-Tactin® AP conjugate
Description	*Strep*-tag®II- and Twin-Strep-tag®-specific monoclonal antibody, unlabeled	*Strep*-tag®II- and Twin-Strep-tag®-specific monoclonal antibody, labeled with horse radish peroxidase	*Strep*-Tactin® protein, labeled with horse radish peroxidase	*Strep*-Tactin® protein, labeled with alkaline phosphtase
Features	- Highly selective - Low background	- Highly selective - Low background	- Sensitive - Fast detection protocols	- Very sensitive - Fast detection protocols
Secondary antibody required (cat. no. 2-1591-001)	Yes, secondary anti-mouse IgG, HRP-conjugated, required	No, direct detection via HRP	No, direct detection via HRP	No, direct detection via AP
Western blot, chromogenic detection	Suited, but not recommended	Recommended	Recommended	Recommended
Western blot, chemiluminescent detection (ECL)	Not recommended	Recommended	Recommended	Not determined
Immunofluorescence, ELISA, FACS*	Recommended	For ELISA only	For ELISA only	For ELISA only
Detects also biotinylated proteins	–	–	+	+
Cat. no.	2-1507-001 (100 µg)	2-1509-001 (75 µg for 25 - 30 Western Blots)	2-1502-001 (0.5 ml)	2-1503-001 (0.5 ml)



The ***Strep*-tag® Protein Ladder** is designed for accurate MW determination on Coomassie Blue stained gels and as positive control on Western blots. As each protein contains the *Strep*-tag®II sequence which is detected by our *Strep*-Tactin® conjugates or *Strep*-tag® specific antibodies, the ladder can also be used for MW determinations on Western blots and serves as a positive control for the various detection systems.
Cat.no. 2-1011-100

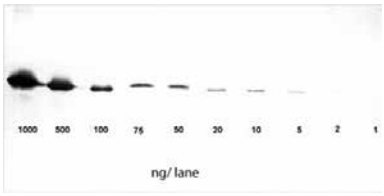
Detection of *Strep*-tag® fusion proteins in Western blots/ELISA

For direct detection of *Strep*-tag® fusion proteins in **Western blots** via **chemiluminescence** reaction, IBA offers:
- *Strep*-Tactin® HRP conjugate (HRP, horseradish peroxidase)
- *Strep*MAB-Classic HRP conjugate.

(no secondary antibody is needed!)

For direct detection of *Strep*-tag® fusion proteins in **Western blots** via **chromogenic** reaction use
- *Strep*-Tactin® AP conjugate (AP, alkaline phosphatase)
- *Strep*-Tactin® HRP conjugate (HRP, horseradish peroxidase)

For protein detection in **ELISA** use
- *Strep*MAB-Classic, unconjugated
- *Strep*-Tactin® HRP conjugate
- *Strep*-Tactin® AP conjugate

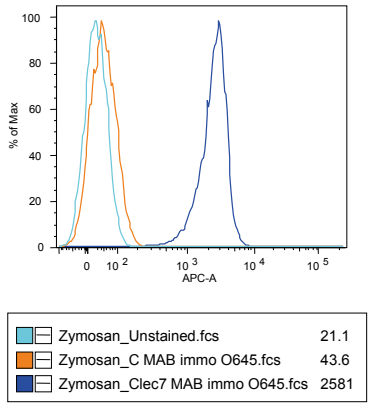


Direct detection of recombinant *Strep*-tag®II GFP in a Western blot using *Strep*-Tactin® HRP conjugate

Detection of *Strep*-tag® fusion proteins in Immunofluorescence/FACS

For the detection of *Strep*-tag® fusion proteins in Immunofluorescence and FACS use
- *Strep*MAB-Classic, unconjugated
- *Strep*MAB-Immo, unconjugated

Use direct labelling with Chromeo™ 488, Chromeo™ 546 or Oyster® 645 conjugated to
- *Strep*-Tactin®
- *Strep*MAB-Classic
- *Strep*MAB-Immo



FACS analysis of Zymosan, stained with *Strep*MAB Immo Oyster 645 against Clec7-Twin-Strep-tag 1:100, 20 min ice
Kindly provided by: Dr. K. Neumann, III Med. Klinik, TUM München

Fluorescent conjugates:

Labelling	*Strep*MAB-Classic	*Strep*MAB-Immo	*Strep*-Tactin®
· Chromeo™ 488 · Chromeo™ 546 · Oyster® 645	50 µg amounts 2-1544-050 2-1550-050 2-1555-050	50 µg amounts 2-1546-050 2-1552-050 2-1557-050	50 µg amounts 2-1542-050 2-1548-050 2-1553-050

MAB and Fab for different species are available:
*Strep*MAB Classic and *Strep*MAB Immo are available as full length antibodies with human, murine or rabbit constant domains.
Fab request at
www.iba-lifesciences.com

2.4 IMMOBILIZATION PLATES

Use for:

- Immobilization assays
- High throughput screens
- Reversible immobilization
- Small-scale purification

Applications

- ELISA
- Antibody or serum screening
- Diagnostic assays
- Protein interaction studies
- Screening of engineered enzymes
- Drug screening

Strep-Tactin® XT coated microplates and 8 well strips

Via Strep-Tactin® XT

Antibody-free option for immobilization of *Strep*-tag® proteins

The ready-to-use Strep-Tactin® XT coated microplates provide the power of the *Strep*-tag® system in a solid-phase, multi-well format for convenient assays and high-throughput screenings for biomolecules tagged with Twin-Strep-tag®. The combination of Strep-Tactin® XT with the Twin-Strep-tag® is highly stable with a $T_{1/2}$ of 13 hours and an affinity in pM range.
The strips of 8 wells are supplied in sets of 12 resulting in a 96-well plate. These 96-well plate configuration is compatible with standard multichannel pipettes, automated plate washers and plate readers. The biomolecules are presented to interaction partners in a uniform manner which results in reliable and highly reproducible assay formats. In addition a beneficial feature of Strep-Tactin® XT coated microplates can be generated.

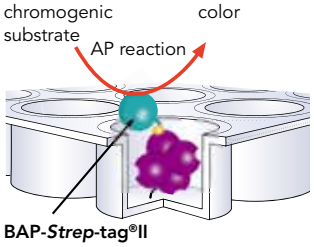
Features:

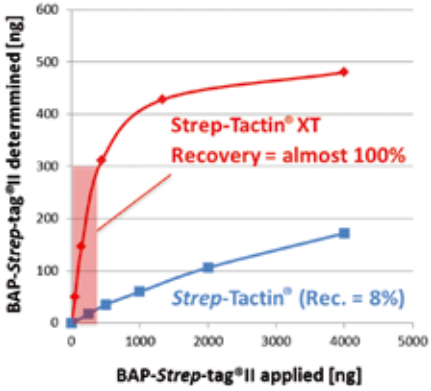
- Oriented binding of recombinant proteins with N-terminal or C-terminal Twin-Strep-tag®
- Minimal non-specific binding
- Minimal coefficients of variation
- High affinity and stability of Strep-Tactin® XT with Twin-Strep-tag®
- Cost-effective
- Reversibility along with high affinity

Related IBA products

Strep-Tactin® XT coated microplates
Cat.no. 2-4101-001

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





Using the MTP assay the binding capacity of Strep-Tactin® XT was examined and compared to *Strep*-Tactin®. For this purpose BAP was fused with *Strep*-tag®II and applied in different amounts onto the *Strep*-Tactin®/Strep-Tactin® XT coated microplates. After washing, the remaining amounts of BAP[1] were measured. Resulting in almost 100 % recovery[2] of BAP-*Strep*-tag®II on Strep-Tactin® XT compared to 8 % recovery on *Strep*-Tactin®.

[1]protein of interest [2]red area

Via *Strep*MAB-Immo

*Strep*MAB-Immo antibody

*Strep*MAB-Immo antibody is the reagent of choice for efficient immobilization of *Strep*-tag®II proteins on solid phases. *Strep*MAB-Immo is a murine, high-affinity *Strep*-tag®II specific monoclonal antibody which is especially suited for stable, mild and oriented immobilization of *Strep*-tag®II fusion proteins. Therefore, the antibody can be coated on different surfaces, e.g. microplates, columns, Biacore CM5 sensor chips or other biochips. The nearly irreversible binding is achieved for fusion proteins carrying a C- or N- terminal (Twin-)*Strep*-tag®. For the latter, the *Strep*-tag® must be extended at the N-terminus by a SerAla linker (recombinant protein-SA-WSHPQFEK or SA-WSHPQFEK-recombinant protein).

*Strep*MAB-Immo coated microplates

This antibody-based highly efficient immobilization of *Strep*-tag® proteins achieves a high wash stability.
*Strep*MAB-Immo coated microplates can be used for efficient, mild and oriented immobilization of SerAla-*Strep*-tag®II fusion proteins for ELISA or other assays used for protein analysis. Also small amounts of such proteins are bound with high efficiency to the microplate and will not elute during the assay due to nearly irreversible binding activity of *Strep*MAB-Immo.

Features:

- Ready-to-use 96-well plates for your own protein assay
- Efficient immobilization of very small amounts of SerAla-*Strep*-tag®II fusion proteins saves your starting material
- High wash stability during the assay
- Irreversible

Related IBA products

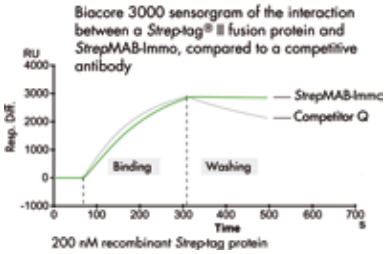
*Strep*MAB-Immo high affinity antibody	Cat.no. 2-1517-001
*Strep*MAB-Immo coated microplates	Cat.no. 2-1521-001

Use for:

Irreversible immobilization of

- *Strep*-tag®II
- Twin-Strep-tag®

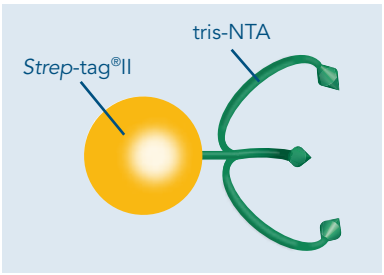
*Strep*MAB-Immo is a high-affinity specific monoclonal antibody for capturing *Strep*-tag® fusion proteins on solid phases



2.5 HIS-TAG PROTEINS IN *STREP*-TAG® QUALITY

Use for:

- Removal of impurities after His-tag purification
- Test *Strep*-tag® without cloning



His-*STREP*PER molecule

His-*STREP*PER

***Strep* your His-tag without cloning**

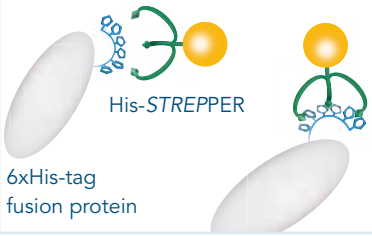
Remove impurities after His-tag purification by adding His-*STREP*PER and thereby convert the His-tag protein into a *Strep*-tag® protein to benefit from the advantages of *Strep*-tag®II – without the need for cloning.

His-*STREP*PER adapter molecule alters a His-tag into a *Strep*-tag®II. It consists of *Strep*-tag®II (SA-WSHPQFEK) conjugated with a nickel charged tris-NTA that tightly binds to the His-tag. Thereby, it gives access to the advantages of the *Strep*-tag® purification system, namely high pure proteins without a time-consuming cloning process.

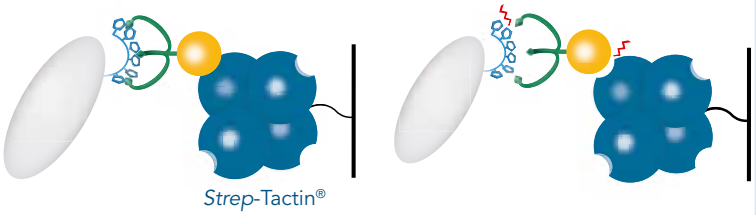
Features:

- Adapter molecule for fast and easy alteration of His-tag fusion proteins into *Strep*-tag®II fusion proteins
- Transfer *Strep*-tag®II advantages (pure & functional proteins) to His-tag proteins
- Cost- and time-effective

His-*STREP*PER binding



Purification via *Strep*-Tactin®



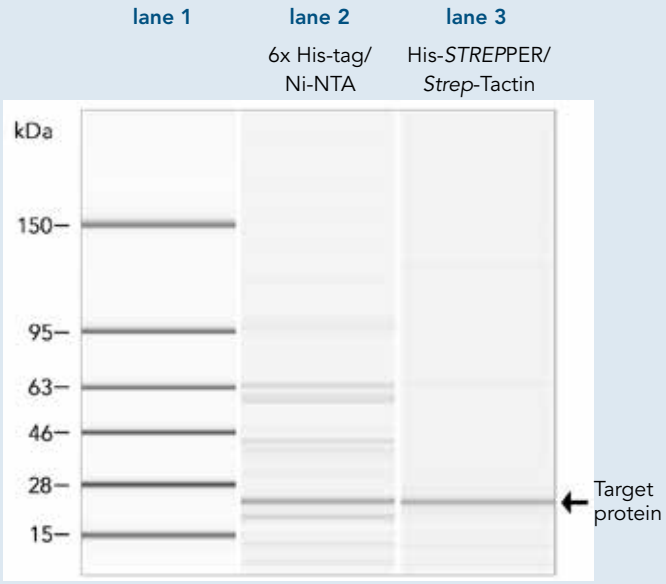
Protein purification example of a 6xHis-tag fusion protein on Ni-NTA resin followed by His-*STREP*PER:*Strep*-Tactin® purification

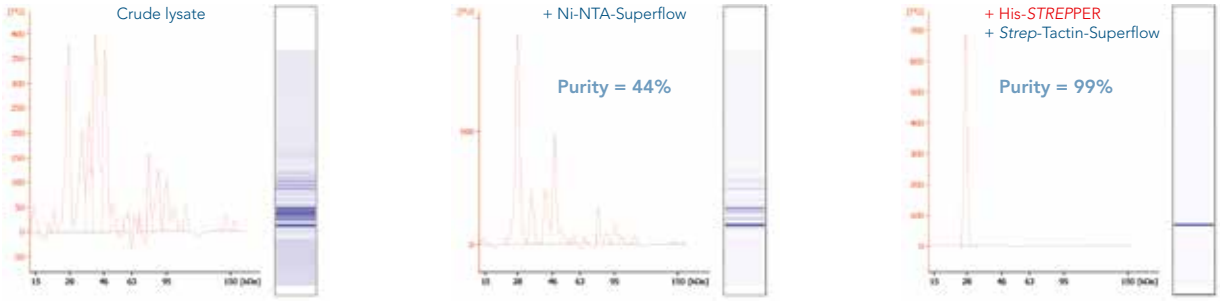
A 6xHis-tag fusion protein from crude bacterial cell extract was applied to a Ni-NTA resin. After washing, the bound protein was eluted using Ni-NTA Elution Buffer (Qiagen, lane 2). Protein purity of the 6xHis-tag fusion protein (arrow) after elution was only 30%. To improve the purity of the eluted protein a further purification step was necessary.

Therefore the elution fraction was dialysed to PBS pH 8.0 and the His-*STREP*PER adapter molecule was added to convert the His-tag into a *Strep*-tag® fusion protein. The elution fraction was applied to *Strep*-Tactin® and the converted protein was eluted with PBS pH 8.0; 2.5 mM desthiobiotin (lane 3).

The addition of His-*STREP*PER and purification via *Strep*-Tactin® led to an increase of the protein purity from 30% to 80 %.

Protein purification analysis was performed with an Agilent Bioanalyzer 2100 system.





His-*STREP*PER purifies 6xHis-tag fusion proteins to higher purity
Purification results for GFP-6xHis using different purification protocols. GFP-6xHis was either purified using the His-*STREP*PER and *Strep*-Tactin® in PBS pH 8 buffer or using Ni-NTA under the same physiological or non-physiological conditions. His-*STREP*PER:*Strep*-Tactin® provides better results than His-tag:Ni-NTA.

Double-tag purification of highly pure full-length proteins

Protein expression is a complex topic with many variables. It is mostly 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 the attachment of two different tags at each terminus of the recombinant protein provides the flexibility to obtain a highly pure and homogenous protein sample.

Important reasons for two different affinity tags on one protein are
- Purification of 100% full length proteins
- Highest purification grades
- Using denaturing or physiological purification conditions

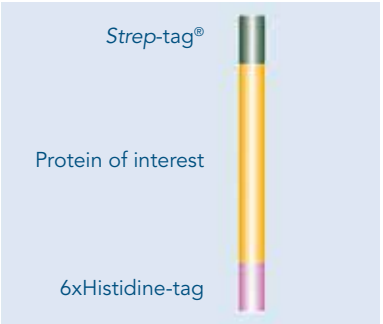
A smart double-tag pair is the combination of *Strep*-tag® and 6xHistidine-tag. Generally, it is recommended to attach one tag to the N-terminus and the other to the C-terminus.

IBA provides two different Ni-NTA supports:
- Ni-NTA Sepharose for purification of 6xHistidine- tag proteins by gravity flow. Cat.no. 2-3202-001
- Ni-NTA Superflow for purification of 6xHistidine-tag proteins by gravity flow and FPLC Cat.no. 2-3207-001

Use for:
Purification of full-length proteins

Strep/6xHistidine-tag Starter Kit with *Strep*-Tactin® Superflow® high capacity

This Starter Kit contains all reagents essential for the native purification of a double-tag protein with *Strep*-tag® and 6xHistidine-tag. The first purification is performed on a Ni-NTA Superflow® cartridge while the second purification uses a *Strep*-Tactin® Superflow® high capacity cartridge, selecting for 6xHistidine-tag and *Strep*-tag®, respectively.
Cat.no. 2-1117-000



	Applications	Purification				Detection	Assay
		Concentrated (cytosolic)	Diluted (secreted)	Batch (magnetic beads)	Denatured (6M urea)	Western Blot ELISA	Immobilization (MTP, chips,...) Incl. optional elution
Strep-Tactin	*Strep*-tag®II	✔	✔	✘	✘	✔	✘
	Twin-Strep-tag®	✔	✔	✔	✘	✔	✔
Strep-Tactin XT	*Strep*-tag®II	✔	✔	✔	✔	✔	✔
	Twin-Strep-tag®	✔	✔	✔	✔	✔	✔

Highlights

- **Twin-Strep-tag® and Strep-Tactin® XT:**
  the 3rd generation of the *Strep*-tag® technology
- **His-*STREP*PER:**
  get His-tag proteins in *Strep*-tag® quality
- **MEXi:**
  the optimized and economic mammalian expression system for high protein yields

Version P84-01-0001

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