

PROTEIN PRODUCTION & ASSAYS

Cloning - Expression - Purification - Detection - Immobilization



The Strep-tag[®]
system

One system for all
protein applications

STREP-TAG® SYSTEM

The Strep-tag® system is a platform providing tools for different recombinant protein applications, such as:

- › Cloning..... p. 4
- › Expression.....p. 6
- › Purification..... p. 8
- › Detection..... p.12
- › Immobilization.....p.14

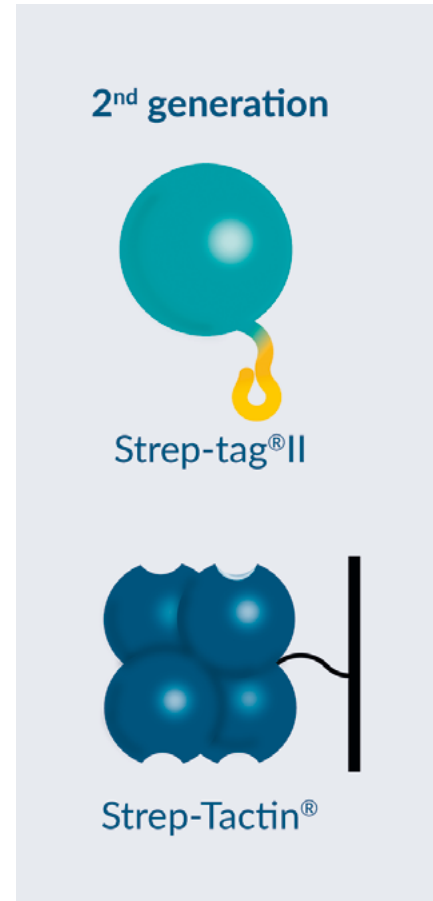
The Strep-tag® system for recombinant protein production is based on one of the strongest non-covalent interactions in nature: the interaction of biotin to streptavidin. Therefore initially a peptide was engineered that binds to the biotin binding site of streptavidin, the so called Strep-tag®. In order to improve the binding affinity of the system, the Strep-tag® as well as the biotin binding site of streptavidin were modified. These modifications lead to Strep-Tactin® and Strep-tag®II.

2nd generation of Strep-tag® system

Strep-tag®II and Strep-Tactin® represent the second generation of the system.

Strep-tag®II is an eight amino acid synthetic peptide (WSHPQFEK), which can be fused to the N- or C-terminus of the protein of interest.

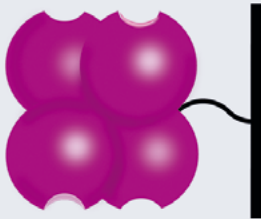
The intrinsic binding affinity of Strep-tag®II towards Strep-Tactin® results in highly specific interactions, which enables the isolation and purification of sensitive proteins in a native state as well as of intact protein complexes.



3rd generation



Twin-Strep-tag[®]



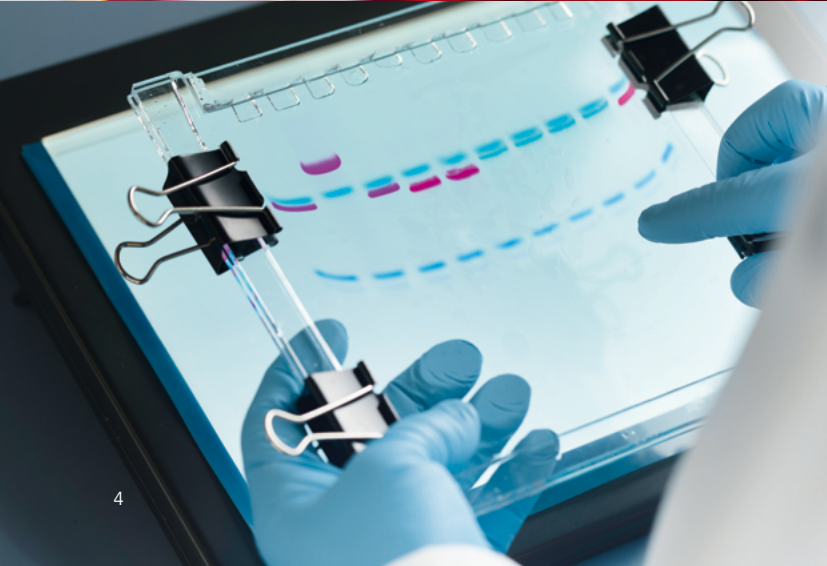
Strep-Tactin[®]XT

3rd generation Strep-tag[®] system

The Strep-tag[®] system was further developed to meet the needs of high affinity applications in certain bioanalytic approaches. Therefore, the Twin-Strep-tag[®] was generated a tandem arrangement of two Strep-tag[®]II motifs in series (28 aa, WSHPQFEK-GGGSGGGSSG-SA-WSHPQFEK). This modification allows a simultaneous binding of both Strep-tags to Strep-Tactin[®] and leads to an avidity effect which increases the binding affinity to Strep-Tactin[®] and especially to the recently developed Strep-Tactin[®]XT by almost 1000-fold compared to Strep-tag[®]II. Strep-Tactin[®]XT is a modified Strep-Tactin[®] variant with an improved binding affinity to Strep-tag[®]II (affinity in nM ranges) and Twin-Strep-tag[®] (affinity in pM ranges).

Benefits of the Strep-tag[®] system

- › Highest protein purity within a single and rapid one-step purification, no need for additional purification
- › Physiological wash buffers to maintain bioactivity and protein:protein interactions
- › Mild and specific elution of tagged target proteins with biotin (Strep-Tactin[®]XT) or desthiobiotin (Strep-Tactin[®])
- › Tolerates a broad range of detergents, chelators, salt or redox conditions
- › Twin-Strep-tag[®]/Strep-tag[®]II fusion to N- or C-terminus



CLONING

IBA offers a set of more than 170 different expression vectors with a variety of different expression features. Thereby, the system provides the flexibility between cloning of a gene of interest (GOI) directly into the chosen expression vector (direct transfer cloning) or into an intermediary vector. This allows rapid and standardized screening via subcloning of the GOI into different expression vectors in parallel (combinatorial cloning via pENTRY vector).

MORE THAN 170 DIFFERENT EXPRESSION VECTORS

Expression hosts

- › *E. coli*
- › Mammalian cells
- › Yeast
- › Baculovirus (for insect cells)

Affinity tags

- › Twin-Strep-tag®
- › Strep-tag®II
- › His-tag
- › GST-tag
- › Flag-tag

Further options

- › Secretion signal
- › Double tag
- › Different promoters and antibiotic resistances for *E. coli*
- › Cloning of more than one GOI into an expression vector

Direct Transfer Cloning

is used for direct cloning of the target sequence into the expression vector

Combinatorial Cloning

is beneficial for easy switch between vectors to test optimal expression conditions (e.g. host, tags,...)

EXPRESSION

MEXi is an IP free mammalian expression system from IBA, which is optimized for Strep-tag® protein production.

Components of the MEXi system

- › pDSG vector series
- › MEXi HEK293E cell line
- › MEXi cultivation medium (MEXi-CM)
- › MEXi transfection medium (MEXi-TM)

Key benefits of MEXi

- › pDSG vectors are small and equipped with oriP for efficient episomal replication
- › Transient expression for time and cost efficient protein production
- › Stable transfection efficiency of MEXi-293E cells throughout a wide range of passages
- › High protein expression levels due to EBV origin of replication (oriP) and EBNA-1
- › Low biotin content in the expression medium for optimal Strep-tag® purification
- › Purification via Strep-tag® system usually results in a protein purity > 95%
- › Especially Twin-Strep-tag® is well suited for a variety of downstream applications e.g. SPR analysis

MEXi is optimized for high throughput mammalian expression and efficient Strep-tag® purification





Strep-tag® and His-tag for protein production in mammalian cells

His-tag is the most commonly used affinity tag, however, it has significant drawbacks for protein production from mammalian cells compared to Strep-tag®. Purification of a His-tag fusion protein from mammalian cells usually leads to an unspecific background and low recovery. Known reasons are the high percentage of host cell proteins containing Histidine residues that bind unspecifically to the purification matrix and the interaction of substances from the cell culture medium. Furthermore His-tag proteins are eluted using high imidazole concentrations, which often interfere with downstream applications. In contrast Strep-tag® purification results in a protein purity of > 95% even from mammalian cells using a physiological buffer composition.

BioLock

Biotin (Vitamin H) is often present in mammalian or insect cell culture media. It binds to the biotin binding site of Strep-Tactin® and therefore prevents the Strep-tag®II or Twin-Strep-tag® fusion protein from interacting with the purification matrix. It is especially important to block free biotin with the economic BioLock solution prior to purification, when the target protein is secreted to the cell culture medium.

Wet Fred

Wet Fred is an application aid for purification of Strep-tag® fusion proteins from large culture volumes via gravity flow columns.

PURIFICATION

The Strep-tag® system is one of the most widely used affinity tags for recombinant protein purification.

For good reasons:

- › Protein purification with unparalleled protein purity (> 95%)
- › Physiological purification conditions preserve protein functionality
- › Time saving due to a fast one-step purification
- › Tolerates variable buffer conditions, e.g. high salts, detergents, metal ions, chelators, reducing agents and denaturing conditions
- › High specificity of Strep-tag®II/Twin-Strep-tag® to Strep-Tactin® and Strep-Tactin®XT provide competitive elution results and low background
- › Re-usability of the robust purification resins
- › Favorable for protein:protein interaction studies due to mild elution conditions and low wash volumes
- › Efficient immobilization via Strep-Tactin®XT (reversible binding) or StrepMAB-Immo (irreversible binding)
- › Removal of the small Strep-tag®II is not necessary since it has a neutral pI and does not influence protein folding or function
- › Availability of a universal detection system for Western blot, ELISA, Immunofluorescence, FACS and more

The Strep-tag® system is perfectly suited for the following protein classes:

- › Metalloproteins
- › Membrane proteins
- › Low abundant proteins
- › Sensitive protein complexes with multiple subunits
- › Bioactive proteins
- › And any other protein

Strep-tag® purification is based on two specificity conferring steps for high purity:

- › Specific binding of the Strep-tag® motif to Strep-Tactin® and Strep-Tactin®XT
- › Competitive elution with desthiobiotin or biotin, respectively

STREP-TAG® PURIFICATION RESINS: STREP-TACTIN® AND STREP-TACTIN®XT

Strep-Tactin® and Strep-Tactin®XT - two variants with different binding properties for variable requirements

The recently developed Strep-Tactin®XT provides an almost 1000-fold stronger binding affinity for Strep-tag®II and Twin-Strep-tag® compared to Strep-Tactin®. Strep-Tactin® should be used for cytosolic proteins. If it comes to more challenging proteins and purification procedures such as diluted or secreted proteins, denaturing conditions or batch purification Strep-Tactin®XT is the resin of choice.

Strep-Tactin® and Strep-Tactin®XT - differences in the purification procedure

There are two main differences to be considered. First, the elution of the target protein from Strep-Tactin® requires desthiobiotin whereas biotin is used for Strep-Tactin®XT. Second, regeneration of Strep-Tactin® is performed with HABA while Strep-Tactin®XT requires low concentrations of magnesium chloride.

Different Strep-Tactin® and Strep-Tactin®XT resins for purification are available in multiple formats.



STREP-TACTIN®XT

Strep-Tactin®XT is the high affinity Strep-Tactin® variant, which enables new applications and an improved performance for the Strep-tag® system:

New applications

- › High-throughput screening
- › Batch purification
- › Denaturing conditions

Improved performance

- › Higher protein yields
 - › Improved purity
 - › Optimized elution profile for highly concentrated target protein
- › High detergent and wash stability
 - › Binding affinity to Twin-Strep-tag® in low pM range

More Strep-Tactin®XT products



STREP-TACTIN®XT 4FLOW®

Efficient purification of large proteins can be challenging. If the agarose has a high concentration, the more difficult it is for large proteins to enter the agarose. As a result, large target proteins are insufficiently immobilized and remain in the flow-through. The solution to this problem is highly specific Strep-Tactin®XT combined with a low concentrated agarose, Strep-Tactin®XT 4Flow®. Strep-Tactin®XT 4Flow® is the resin to start with due to particular applicability even for low abundant or challenging proteins. In addition, a further increase in protein yield can be achieved through the high capacity variant of Strep-Tactin®XT 4Flow®.

Use Strep-Tactin®XT 4Flow® to get the most out of your cell lysate



DETECTION

Twin-Strep-tag® and Strep-tag®II fusion proteins can be detected in western blot analysis, FACS, immunocytochemistry, immunohistochemistry and ELISA. For this IBA provides various conjugates to Strep-Tactin® as well as to monoclonal antibodies, StrepMAB-Immo and StrepMAB-Classic.

Strep-Tactin®	Strep-Tactin®XT	StrepMAB-Classic	StrepMAB-Immo
AP	DY-488	DY-488	DY-488
HRP	DY-549	DY-549	DY-549
unconjugated	DY-649	DY-649	DY-649
		HRP	
		unconjugated	

Two different detection systems are available:

- › Monoclonal antibodies, conjugated or unconjugated
- › Strep-Tactin®XT conjugates





STREP-TACTIN®XT CUSTOMER STATEMENTS

"I was able to perform my protein purification from glycerol stock to pure protein in less than 48 hours! This normally takes two weeks. Strep-Tactin®XT has saved me a lot of time!"

Dr. Jessica van Wonderen,
Post-Doctorate Researcher,
University of East Anglia, UK

"Strep-Tactin®XT led to the final breakthrough in our current project"

Dr. Sabine Suppmann,
Recombinant Protein Production,
MPI of Biochemistry,
Martinsried, Germany

Strep-Tactin[®]XT and
Twin-Strep-tag[®] for high
affinity applications

The only affinity
tag that binds in
pM range

IMMOBILIZATION

IBA provides with Strep-Tactin[®]XT an antibody-free option for immobilization, which allows oriented binding of recombinant proteins with N- or C-terminal Strep-tag[®]II or Twin-Strep-tag[®]. This cost effective alternative provides the highest binding affinity among peptide based affinity systems.

StrepMAB-Immo is a high affinity, monoclonal Strep-tag[®]II antibody and can also be used for immobilization. However, when Strep-tag[®]II is used the motif needs to be extended at the N-terminus by a SA motif (SA-WSHPQFEK). Immobilization with this antibody is irreversible.

Strep-Tactin[®]XT

The best results for immobilization and assays of recombinant proteins can be achieved by using Strep-Tactin[®]XT in combination with Twin-Strep-tag[®].

- › High affinity in pM range ($T_{1/2} = 13h$)
- › Reversibility due to addition of Biotin

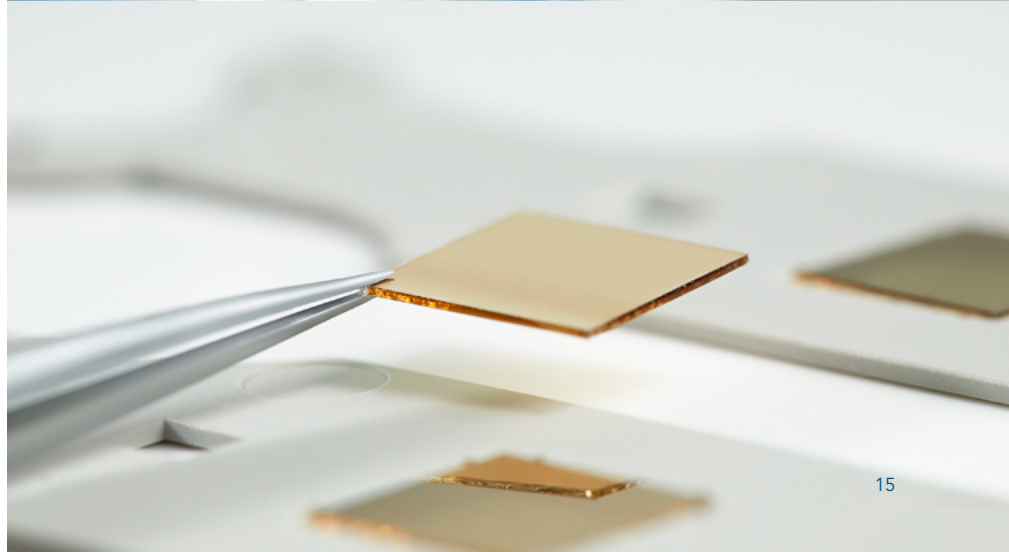


Strep-Tactin[®]XT Immobilization plate

Twin-Strep-tag® Capture Kit

This kit is intended for site-directed, reversible capture of Twin-Strep-tag® fusion proteins (ligand) on Strep-Tactin®XT (capture molecule) for biomolecular interaction analysis using Biacore™ SPR systems.

- Chip can be regenerated easily by an established procedure
- Stable long-term measurements



Technical support

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THE STREP-TAG® SYSTEM: A UNIVERSAL TOOLBOX



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