Protein Production & Assays

The Strep-tag® technology – One affinity tag for all protein applications

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Protein purification is one of the main applications in biotechnology, as it allows to analyze the target protein without influences from other molecules. Affinity chromatographic protein purification is based on the binding affinities between different molecules. However, the purification process is influenced by the target protein class and properties, buffer conditions, and expression host. IBA´s flexible Strep-tag® system enables the purification of functional target proteins with high purity and yield in a fast and easy way and is therefore ideally suited for protein crystallization.

THE STREP-TAG® SYSTEM

CLONING & EXPRESSION
Vectors with multiple tags · Various expression hosts · Optimized mammalian expression system

PROTEIN PURIFICATION
Easy purification cycle · Flexible buffer composition · All protein classes possible · Crystallization without tag cleavage

ANALYSIS
Sensitive detection agents · Efficient protein immobilization (SPR / BLI)

EXPRESSION HOST

The best choice for protein expression for structural analysis is an expression host that closely resembles the source organism of the protein. The Strep-tag® system offers a diverse range of expression vectors that are compatible with a variety of hosts. Furthermore the short Strep-tag® sequence can easily be cloned into any vector of choice independent of the expression host.

Bacteria
E. coli, C. saccharolyticum, Thermosynechococcus elongatus

Fungi
Yeast, M. oryzae

Mammalia
HEK, HeLa, mice (Tc6-3), CHO

Insect cells
Baculovirus

Plants
Arabidopsis, Tobacco, Phaseolus

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PROTEIN CLASS

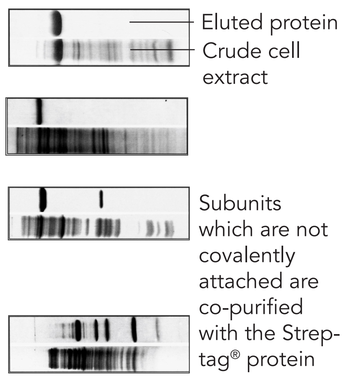
Protein class, localization and specific domains are all characteristics that will influence the choice of purification system.
Even challenging protein classes, including metalloproteins, large proteins or protein complexes can be successfully purified via the Strep-tag®.

Metalloenzymes
E. coli alkaline phosphatase

Large proteins
Oat phytochrome A, 120 kDa

Heterodimeric proteins
Helicobacter pylori urease
Heavy chain with Strep-tag®
Light chain co-purified

Multimeric membrane proteins
Paracoccus denitrificans cytochrome c oxidase
Recombinant antibody fraction with Strep-tag®
Membrane protein consisting of 4 subunits co-purified



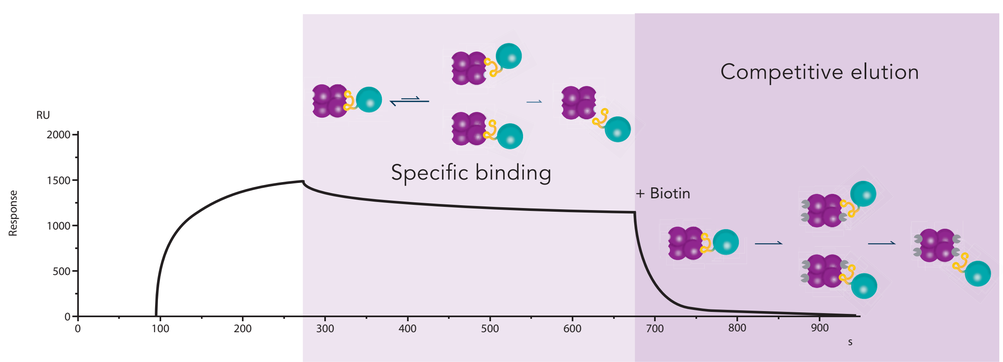
FLEXIBLE BUFFER CONDITIONS

To natively purify the target protein, the buffer components have to be adjusted accordingly. Unlike other purification systems, the Strep-tag® is compatible with a wide variety of common buffers and additives, as well as a broad pH range.

Reagents	Strep-tag® compatibility
Detergents For cell wall lysis, purification of membrane proteins	
Reducing agents To reduce disulfide bonds, as an enzyme stabilizer	
Chelating agents For eliminating cations, to inhibit protease activity	
Buffer components To adjust the pH for native protein folding	
Tris-HCl	✓
PBS	✓
HEPES	✓
Other additives	
NaCl	5 M
Imidazole	250 mM
Glycerol	Max. 25 %

HIGH SPECIFICITY LEADS TO HIGH PURITY

- Highly specific binding of the (Twin-)Strep-tag® to Strep-Tactin®XT
- Avidity effect of Twin-Strep-tag® increases affinity – robust binding
- Specific competitive elution through biotin



TARGET PROTEIN REQUIREMENTS

- **High yield**
- **High purity**
- **Naturally folded protein**

BENEFITS OF THE STREP-TAG® SYSTEM

- Strep-tag® purification is fast and easy
- Highly pure proteins in a single step
- Optimization of the purification process is not required
- Robust system allows adjustment of buffers to protein requirements
- Strep-tag® resins can be regenerated and reused several times

Simple purification procedure

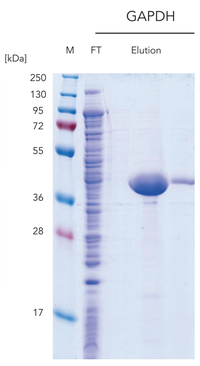
1. Equilibration
Equilibrate with buffer W

2. Sample application
Load sample

3. Wash
Wash with buffer W

4. Elution
Elute protein with biotin

Protein purification workflow



SDS Gel of one Twin-Strep-tagged protein after purification with Strep-Tactin®XT resin. Strep-tag® purification offers a purity of up to 95%.

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