One-STrEP analysis of protein:protein-interactions

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Strep-tag® and One-STrEP-tag

The *Strep*-tag® was originally selected from a random library for specific streptavidin binding activity enabling purification of corresponding fusion proteins on streptavidin affinity columns. Binding reversibly to the same pocket where the natural ligand D-biotin is complexed, elution of the bound recombinant protein is effected by competition with D-biotin.

The system was systematically optimised, including development of the optimised *Strep*-tag®II for N- or C-terminal as well as protein internal fusion and engineering of a streptavidin variant with improved binding capacity, dubbed *Strep*-Tactin®.

A particular benefit of *Strep*-tag®II is that it has a neutral amino acid composition and that it does not hamper protein folding or secretion, nor does it interfere with protein function. *Strep*-tag® enables purification of recombinant proteins to over 99% purity in a single step from crude lysates. The extraordinary purification factors are based on i) very low tendency of *Strep*-Tactin® to bind other proteins non-specifically, ii) highly specifc *Strep*-tag®II:*Strep*-Tactin® interaction and iii) specific competitive elution with minute amounts of desthiobiotin in the physiological wash buffer.

Suitability for protein:protein interaction studies

Strep-tag® technology was used from the beginning to purify intact protein complexes in a preparative manner, even if just one subunit carries the tag. The development of a tandem arrangement of two *Strep*-tag®II sequences, dubbed One-STrEP-tag, improved performance by increasing purification yields of poorly expressed protein complexes.

Protein:protein-interactions (PPI) govern almost all important processes in living organisms. Thus, their rapid and accurate determination and investigation is a major challenge in life sciences. IBA provides optimal solutions with its different determination systems for protein:protein-interaction analysis.

The One-STrEP™ system (one-step purification with One-STrEP-tag on *Strep*-Tactin®) is recommended for getting started. It needs one tag and one purification step only and is validated for eukaryotes and prokaryotes. Due to its excellent performance, this method yields a favourable signal-to-noise ratio in most cases. Mild elution and fast washing allow the isolation of even weakly interacting preys.

If the One-STrEP system provides suboptimal data the 'One-TAP' system (One-tag tandem affinity purification with One-STrEP-tag on *Strep*-Tactin® and *Strep*MAB-Classic) extends the options of the One-STrEP system by adding a second independent purification step with the same tag. Two different purification steps may better discriminate specific from non-specific binding but bears the risk of losing weakly interacting partners.

Two different tags increase the risk of non-specific binding or interference with the native conformation of the bait necessary for an effective binding of associated proteins.Therefore the 'Two-TAP' system (Two-tag tandem affinity purification with One-STrEP-tag on *Strep*-Tactin® and FLAG®-tag on M2 mAb) is only recommended when poor results with the One-STrEP™ or 'One-TAP' approach are obtained.

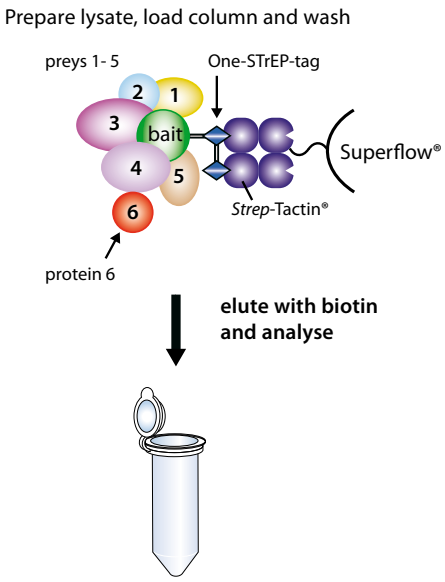
In addition to these non-covalent capture methods of potential preys, SPINE (Strep-protein interaction experiment with *Strep*-tag®II) adds the possibility to covalently link the preys to its bait by formaldehyde crosslinking. This linkage is achieved in the living organism enabling a time resolved snapshot of interacting proteins. SPINE is currently only validated in prokaryotes.

StarGate® for bait cloning

This novel cloning system is the perfect tool for efficient screening and fast identification of the optimal tag for PPI investigation with a given bait. Once the bait protein is cloned into the donor vector, a large selection of acceptor vectors for its expression with different tag arrangements is available.

For PPI analysis we recommend acceptor vectors equipped with the following features: (included in PPI sets)

- One-STrEP-tag at N- and C-terminus (for One-STrEP, One-TAP, SPINE)
- One-STrEP/ FLAG®-tag double tag, N- and C-terminal (for Two-TAP)
- *Strep*-tag®II at N- and C-terminus (for SPINE)



One-STrEP

The One-STrEP system isolates protein complexes by a single affinity purification step on Strep-Tactin® Superflow®, including short washing only, thereby enabling co-purification of weakly associated preys. Physiological buffers are used throughout the purification process and elution is performed with minute concentrations of biotin in the same buffer.

Advantages

- Only one tag, only one purification step
- Fast and easy purification conserves even weak protein:protein-interactions
- Good signal-to-noise ratio
- Mild elution conditions conserve functional structures of protein complexes
- Convenient and highly flexible bait cloning procedure available (StarGate®)

Disadvantages and risks

- In some cases background due to non-specifically binding proteins may occur

Catalogue No	Alt. No	Description
IB21121001	2-1121-001	One-STrEP set for mammalian cells
IB21121002	2-1121-002	One-STrEP set for *E. coli* cells