Strep-tag® protein purification system

IBA

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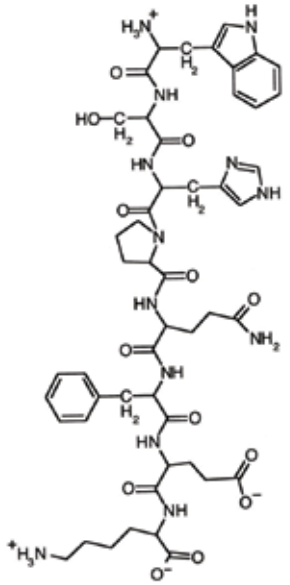
- Short tag does not influence the protein
- Rapid one-step purification under physiological conditions
- Unsurpassed purity and bioactivity

The *Strep*-tag® purification system is based on the highly selective and easily controllable interaction between the *Strep*-tag®II peptide and the biotin binding site of a specially engineered streptavidin called *Strep*-Tactin®. *Strep*-tag®II binds *Strep*-Tactin® nearly 100 times tighter than streptavidin, but elutes under gentle, physiological conditions. Rapid, one-step affinity purification results in active fusion proteins of highest purity. Physiological buffers like PBS in combination with a wide range of detergents, chelators, salt, and redox conditions can be used.
The competitive elution with desthiobiotin, an inexpensive, reversibly binding and stable analog of biotin, enables unparalleled purification factors. The system is safe and easy to use; column regeneration and activity status are visualised by a colour change on the purification column.
A particular benefit of *Strep*-tag®II is its neutral amino acid composition that 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 specific *Strep*-tag®II:*Strep*-Tactin® interaction and iii) specific competitive elution with minute amounts of desthiobiotin. Moreover, extreme stability of *Strep*-Tactin® is the basis of robust affinity resins.



Strep-tag® II
NH_2-WSHPQFEK-COOH

The Strep-tag® protein purification cycle

Purification procedure under physiological conditions.
The purification of *Strep*-tag®II fusion proteins can be performed under nearly physiological conditions, e.g., in PBS buffer and for elution in PBS/2.5mM desthiobiotin buffer:

Steps 1 and 2: The cell lysate is added to the column. Once the tagged protein has bound specifically to Strep-Tactin® the host proteins are washed away rapidly with small amounts of physiological wash buffer.

Step 3: Then, bound *Strep*-tag®II protein is gently eluted by adding wash buffer containing additionally 2.5mM desthiobiotin which specifically competes for the biotin binding pocket.
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. Next to the specific binding of *Strep*-tag® to *Strep*-Tactin®, this is the second specificity conferring step of this purification procedure, yielding extremely high protein purity.

Steps 4 and 5: 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 colour turns to red conveniently indicating the regeneration and activity status of the column.

Step 6: HABA can be removed simply by adding wash buffer. Once the red colour has disappeared the column can be re-used. *Strep*-Tactin® resin can be regenerated and re-used 3 to 5 times without loss in performance.

Strep-Tactin® resins

For *Strep*-tag® affinity purification.
Several *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®, the new *Strep*-Tactin® Superflow ® high capacity and *Strep*-Tactin® MacroPrep® can also be used for low pressure, FPLC and HPLC applications, and *Strep*-Tactin® POROS® (20 and 50) for FPLC and HPLC chromatography. In addition, Superflow is especially suited for increased flow rates and for the purification of large protein complexes.

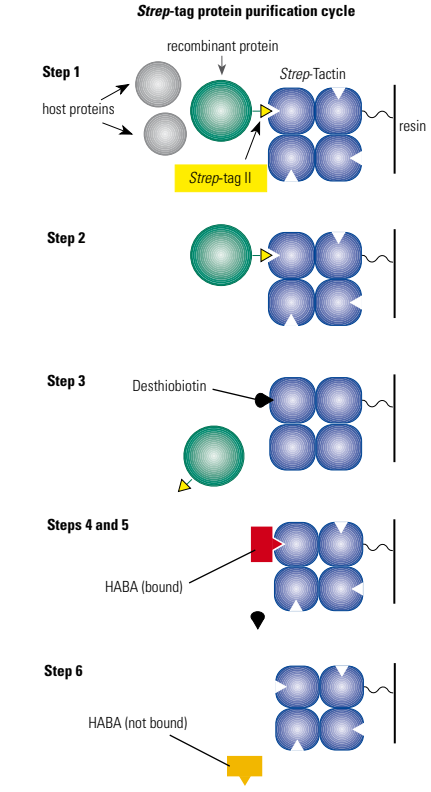
H-PR ('highly pressure resistant') cartridges (No. 5)

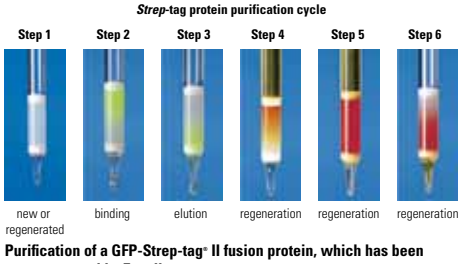
The H-PR cartridges are primarily designed for use with chromatography workstations using 10 to 32 fittings (e.g., HPLC and AKTA™). They can, however, also be operated with other workstations, syringes or peristaltic pumps by use of common adapters. Since column housings are highly pressure resistant (up to 20 bar), H-PR cartridges can be used with a flow restrictor. Cartridges can be connected in series to enlarge capacity.

Strep-Tactin® resins

Catalogue No	Alt. No	Type	Capacity	Flow rate
IB21201010	2-1201-010	*Strep*-Tactin® Sepharose® Exclusion limit: 3 x 10^7 Da	50 to 100nmol/mL	Up to 30cm/hr
IB21206010	2-1206-010	*Strep*-Tactin® Superflow® Exclusion limit: 6 x 10^6 Da	50 to 100nmol/mL	Up to 300cm/hr
IB21208010	2-1208-010	*Strep*-Tactin® Superflow®, high capacity Exclusion limit: 6 x 10^6 Da	150 to 500nmol/mL	Up to 300cm/hr
IB21505010	2-1505-010	*Strep*-Tactin® MacroPrep® Exclusion limit: 1 x 10^6 Da	50 to 100nmol/mL	Up to 300cm/hr
IB21203010	2-1203-010	*Strep*-Tactin® POROS® 20	40 to 80nmol/mL	300 to 500cm/hr
IB21205010	2-1205-010	*Strep*-Tactin® POROS® 50	40 to 80nmol/mL	300 to 500cm/hr

entry continued





Purification of a GFP-Strep-tag® II fusion protein, which has been overexpressed in *E. coli*.
1. New or regenerated column.
2. Specific binding of GFP-*Strep*-tag® II fusion protein to *Strep*-Tactin® Sepharose® column while host proteins are rapidly washed away with small amounts of physiological buffer.
3. *Strep*-tag® protein is eluted due to addition of the specific competitor 'desthiobiotin'.
4 to 6. Column regeneration: desthiobiotin is displaced by the yellow solution containing HABA, which turns red once complexed with Strep-Tactin®. HABA is then removed by washing buffer and the column is ready for the next purification run.