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Protein Production &
Assays

Purification of Strep-tag® fusion proteins under denaturing conditions

A comprehensive manual

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For research use only

Content

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1 Introduction

Strep-tag® system overview

The Strep-tag®:Strep-Tactin® system is one of the most widely used affinity chromatography systems for the purification of highly pure proteins, as well as protein detection and immobilization. Based on the well-known binding of biotin to streptavidin (Fig. 1) we developed a peptide that is capable of binding to the biotin binding pocket of streptavidin when fused to recombinant proteins. This short peptide, which serves as purification tag, consists of only eight amino acids (sequence: WSHPQFEK) and was named Strep-tag®II. To optimize binding to Strep-tag®II, also streptavidin has been engineered to obtain Strep-Tactin®. Constant research led to further developments and finally resulted in the 3rd generation of the Strep-tag® system: **Strep-Tactin®XT and Twin-Strep-tag®**.

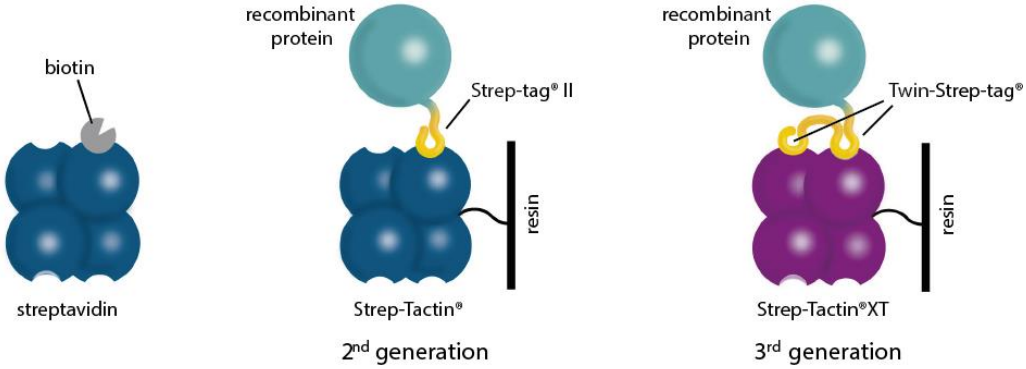


Fig. 1: Schematic view of the Strep-tag® core technology

The Strep-tag® II can be fused to the protein at both the N- or C-terminus and binds with high selectivity to Strep-Tactin®. Due to its chemically balanced amino acid composition the effect on the recombinant protein is negligible. This technology allows one-step purification of recombinant proteins under physiological conditions, thus preserving their bioactivity.

Based on the proprietary Strep-tag® technology the **Twin-Strep-tag®** was developed which is a sequential arrangement of two Strep-tag®II sequences. This tag enables the same mild and rapid purification as Strep-tag®II but, due to its avidity effect, has an increased affinity for Strep-Tactin®. Like Strep-tag®II, the Twin-Strep-tag® tolerates diverse buffer conditions and additives (high salt, detergents, reducing agents, metal ions and chelating agents) making it a universal tag for varying protein properties.

IBA's 3rd generation Strep-tag® system is based on the novel **Strep-Tactin®XT** Superflow® resin in combination with the aforementioned Twin-Strep-tag®. Strep-Tactin®XT has a **binding affinity in low pM ranges** for the Twin-Strep-tag® still maintaining reversible binding, mild recovery of immobilized proteins and unparalleled high purity (> 95 %).

The development of Strep-Tactin®XT enables new applications in the field of high throughput screening, batch purification and protein interaction studies. Due to its altered characteristics compared to Strep-Tactin®, Strep-Tactin®XT can be used with up to 6 M urea and therefore allows purification under **denaturing conditions**. It is recommended to use Strep-Tactin®XT in combination with Twin-Strep-tag® for this approach.

2 Protocol for Strep-tag® purification under denaturing conditions

Recommended Buffers/Solutions	Concentration of ingredients	Notes
Strep-Tactin®/XT Wash Buffer; Buffer W + 6 M urea	100 mM Tris/HCl pH 8.0 150 mM NaCl 1 mM EDTA 6 M urea	It is recommended to work without EDTA when metallo-proteins are purified
Strep-Tactin®XT Elution Buffer with Biotin; Buffer BXT + 6 M urea	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 50 mM biotin 6 M urea	
5x SDS-PAGE sample buffer	250 mM Tris/HCl, pH 8.0 25 % glycerol 7.5 % SDS 0.25 mg/ml bromophenol blue 12.5 % v/v mercaptoethanol	

2.1 Preparation of cleared lysate from bacterial pellet

Protocol

1. **Thaw the cell pellet for 15 minutes on ice and resuspend with Buffer W + 6 M urea at 5 ml per gram wet weight.**

2. **Stir cells for 15-60 minutes at room temperature or lyse them by gently vortexing, taking care to avoid foaming.**
   Lysis is complete when the solution becomes translucent. Stirring time can be extended if the sample is still turbid after 60 min.

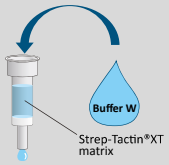
3. **Centrifuge lysate at 10,000 x g for 20-30 minutes at room temperature to pellet the cellular debris.**
   Save supernatant (cleared lysate).

4. **Take a 20 µl sample from supernatant. Add 5 µl 5x SDS-PAGE sample buffer and store at -20°C until SDS-PAGE analysis.**

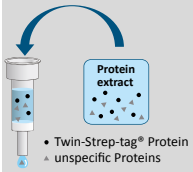
5. **Cleared lysate is ready for application onto Strep-Tactin XT®.**

2.2 Purification of Strep-tag® fusion proteins under denaturing conditions

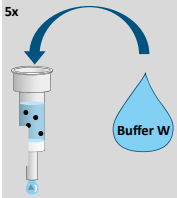
Protocol



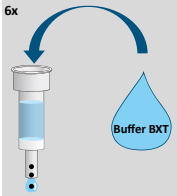
1. **Remove top cap from column first, then the cap at the outlet of the column**. If the caps are removed in reverse order, air may enter the column bed. Remove storage buffer. The column cannot run dry under gravity flow.
   Equilibrate the Strep-Tactin®XT column with 2 CV (column bed volume) Buffer W + 6 M urea (100 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA).



2. **Add cleared lysate to the column.**
   The volume of the lysate should be in the range of 0.5 and 10 CVs (see Table 1). Frozen lysates have to be centrifuged prior to application (18.000 x g, 5 min, 4°C) in order to remove any aggregates that may have formed.

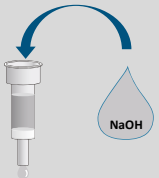


3. **Wash the column with 5 CV Buffer W + 6 M urea, after the cell extract has completely entered the column.**
   Collect the wash fractions (1 CV each) and optionally save 2 µl of each subsequent wash fraction for application on an analytical SDS-PAGE.



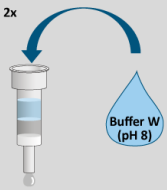
4. **Add 6 times 0.5 CVs Buffer BXT + 6 M urea and collect the eluate in 0.5 CV fractions.**
   Option: To get high protein concentrations in one fraction add 0.6 CV as elution fraction 1 (E1), then 1.6 CV (E2) and finally 0.8 CV (E3). Main protein content should be in fraction E2. 20 µl samples of each fraction can be used for SDS-PAGE analysis.

2.3 Regeneration of Strep-Tactin®XT Superflow® Resins



1. **Wash the column with 8 CV of 10 mM NaOH for regeneration**

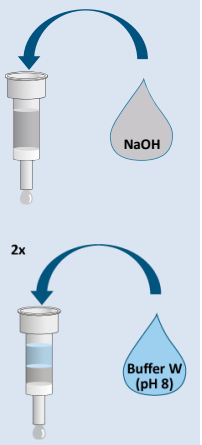
   Always use freshly prepared 10 mM NaOH.
   Strep-Tactin®XT Superflow® resin cannot be regenerated using HABA (Buffer R). However, after treatment with NaOH, operability can be confirmed by application of Buffer R which induces an orange-shift in case of a successful regeneration.



2. **Immediately remove NaOH by adding 2x 4 CV Buffer W (pH 8.0).**

   Column can be stored in Buffer W at 4°C

2.4 Regeneration of Strep-Tactin®XT Superflow® High Capacity Resins



1. **Wash the column with 15 CV of 10 mM NaOH for regeneration**

   Always use freshly prepared 10 mM NaOH.
   Strep-Tactin®XT Superflow® resin cannot be regenerated using HABA (Buffer R). However, after treatment with NaOH, operability can be confirmed by application of Buffer R which induces an orange-shift in case of a successful regeneration.

2. **Immediately remove NaOH by adding 2x 4 CV Buffer W (pH 8.0).**

   Column can be stored in Buffer W at 4°C

2.5 Recommended volumes for working with Strep-Tactin®XT columns

Column bed volume (CV)	Washing buffer volume	Elution buffer volume
0.2 ml	5 x 0.2 ml	6 x 0.1 ml
1 ml	5 x 1 ml	6 x 0.5 ml
5 ml	5 x 5 ml	6 x 2.5 ml
10 ml	5 x 10 ml	6 x 5 ml

Table 1. Recommended buffer volumes for chromatography on Strep-Tactin®XT columns
Adjust volume of cleared lysates according to binding capacity of the column (please refer to the appropriate data sheet) and apply the lysate as concentrated as possible in the recommended volume range. Note that these volumes are average values which can be different for certain proteins.

3 Related products

Cat. No.	Product
2-1003-100	Strep-Tactin®/XT Wash Buffer; 10 x Buffer W; 100 ml
2-1041-250	Strep-Tactin®XT Elution Buffer with Biotin; Buffer BXT; 250 ml

Please download always an up-to-date version of this protocol from:
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