PreCap STarm Streptactin

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1. Product Description

Strep-tag is a widely used affinity tag in protein purification systems. It consists of two types Strep-tag II and Twin Strep-tag II. Strep-tag II is a short peptide tag consisting of 8 amino acids (WSHPQFEK) that can be fused to proteins as an N-terminal or a C-terminal tag with minimal effect on recombinant proteins. The further improved Twin Strep-tag II is a sequence of two Strep-tag II sequences in a sequential order (linked by internal amino acids), and this tag is capable of gentle and rapid purification like Strep-tag II. The two tags are free to bind either of the ligands in **Streptactin** and **STarm Streptactin**. Tag/ligand binding depends on the desired binding strength and application. The affinity of these two tags to **Streptaction** and **STarm Streptaction** is in the μg/ml range, a high affinity that is not achieved by any other existing affinity labeling system. In addition, this flexibility of combining tags and ligands allows purification of recombinant proteins under physiological conditions.

Strep-tag labeling technology can be used to purify functional strep-tagged proteins from a variety of expression systems, including baculoviruses, mammalian cells, yeast, and bacteria. In general, both of these tags do not interfere with the folding or biological activity of the target proteins, do not react with heavy metal ions, do not have ion-exchange properties, and do not cause protein aggregation. Therefore, there is no need to remove Strep-tag II and Twin Strep -tag II after purification.

The ligand protein for **STarm Streptactin Beads 4FF** is Streptactin Mutant coupled to highly cross-linked 4% agarose microspheres. Low concentrations (50 μmol/L) of D-Biotin in the sample do not affect the binding of the target protein to STarm Streptactin Beads 4FF. The product can be regenerated with an equilibrium solution after use or cleaned with 10 mM NaOH. The characteristics are shown in Table 1.

PreCap STarm Streptactin is one of a range of prepacked, ready-to-use columns for affinity chromatography. It is packed with 1 ml and 5 ml of **STarm Streptactin Beads 4FF**. Five different packing sizes are available. **PreCap STarm Streptactin** has the standard interface and can be adapted to all kinds of chromatography system, such as ÄKTA. It is fast, simple and easy operation.

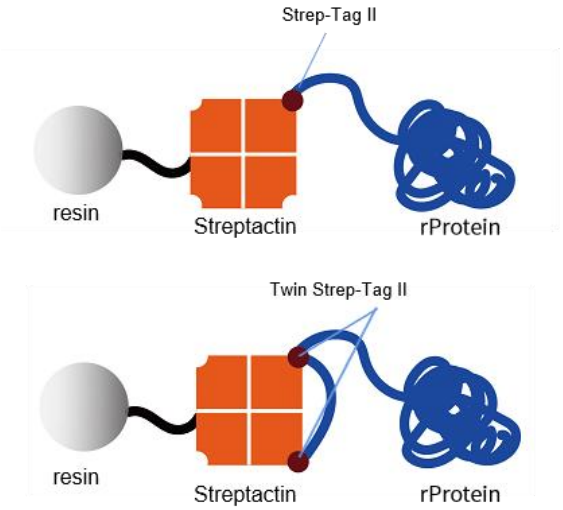

Figure 1. Schematic diagram of Strep-tag Ⅱ and Twin Strep -tag Ⅱ combined with Streptaction

Table 1. Characteristics of **PreCap STarm Streptactin**

Item	Description
Matrix	Highly cross-linked 4% agarose beads
Ligand	Streptactin Mutant
Capacity (/ml medium)	4 mg twin Strep-tag II fusion proteins
Particle Size (μm)	45-165 μm
Maximum Flow Rate	300 cm/h
Storage Buffer	1×PBS containing 20% ethanol
Storage Temperature	2-8℃

Table 2. Chemical compatibilities for **PreCap STarm Streptactin**

Reagent	Contact time
6 M Guanidine hydrochloride 8 M Urea 2 M NaCl	2 hours
50 mM DTT 50 mM β-Mercaptoethanol 1 mM TCEP 0.1% SDS 2% Triton X-100 2% Tween 20 0.25 M imidazole 25% glycerol 1 M $(NH_4)_2SO_4$ 0.1 M $MgCl_2$ 0.1 M $CaCl_2$	1 hour

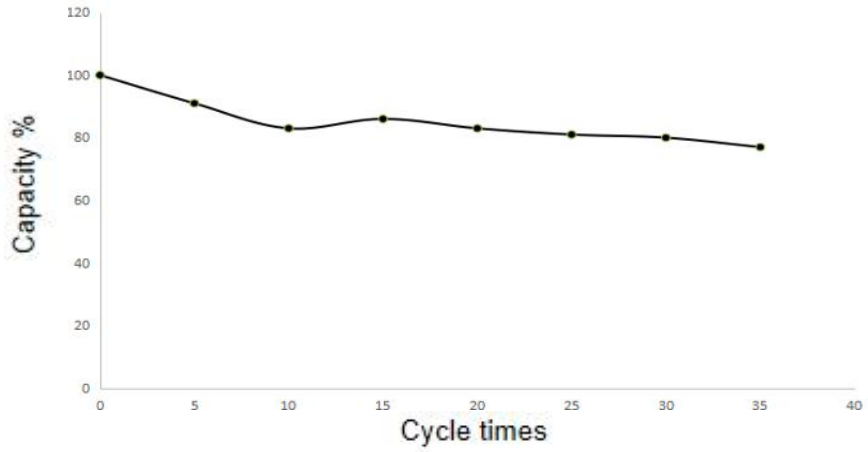

Figure 2. CIP Cleaning of STarm Streptactin Beads 4FF

2. Purification Procedure

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended to filter the buffers by passing them through a 0.22 μm or 0.45 μm filter before use.

LB medium: 10 g/L peptone, 5 g/L yeast powder, 5 g/L NaCl

Antibiotic: 50 mg/ml Kana

Induction agent: 1 mol/L IPTG

2×SDS-PAGE Loading Buffer: 100 mM Tris-HCl, 20% glycerol, 4% SDS, 0.1% bromophenol blue, 200 mM DTT, pH 6.8
Binding/Wash buffer: 100 mM Tris-HCl, 150 mM NaCl, 1mM EDTA, pH 8.0 or PBS
Elution Buffer: 1-5 mM D-Biotin in binding buffer
Regeneration Buffer: 10 mM NaOH

2.2 Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done either by diluting the sample with binding buffer or by buffer exchange. It is recommended to filter the sample solution by passing them through a 0.22 μm or 0.45 μm filter before use.

2.3 Sample Purification

STarm Streptactin Beads 4FF binds Strep-Tag II in three general steps: Binding, Wash, and Elution (Figure 3).

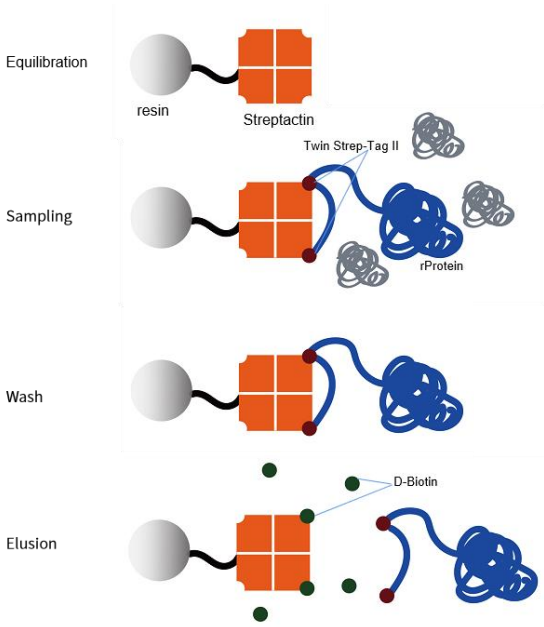

Figure 3. Schematic diagram of STarm Streptactin Beads 4FF purification

PreCap STarm Streptactin is a prepacked, ready to use column. The prepacked column provides fast, simple and easy separations in a convenient format.

1) Fill the syringe or pump tubing with distilled water. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the stopper at the column outlet and connect the column to the chromatographic system.
2) Wash the column with 3-5 column volumes of distilled water.
3) Equilibrate the column with at least 5 column volumes Lysis Buffer.
4) Apply the pre-treated sample, using a Loop fitted to the connector or by pumping it onto the column.
5) Wash with Wash Buffer until the absorbance reaches the baseline or no material appears in the effluent (Generally at least 10-15 column volumes).
6) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

2.4 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or western blot.

2.5 PreCap STarm Streptactin Preservation

PreCap STarm Streptactin should be regenerated once after each use to remove D-Biotin bound to the media to ensure consistent results, as specified:

- 3 column volumes of deionized water;
- 5 -10 column volumes of 10 mM NaOH;
- 3 column volumes of deionized water, and then the pre-packed columns were exchanged into 1×PBS containing 20% ethanol and stored at 2-8℃.

3. Cleaning-in-Place

In general, **PreCap STarm Streptactin** is well suited for reuse several times. When reduced performance or an increase in back-pressure are noted, you need to clean the medium with the solutions as follows:

- 3 column volumes of deionized water;
- 5 -10 column volumes of 0.1 M NaOH;
- 3 column volumes of deionized water, and then the pre-packed columns were exchanged into 1×PBS containing 20% ethanol and stored at 2-8℃.

4. Troubleshooting

Problem	Probable Cause	Solution
Back pressure exceeds 3 bar	Filters are clogged	Clean or replace the filter.
	Column is clogged	Cleaning in place (part 3).
		Filter the sample solution by passing them through a 0.22μm or 0.45 μm filter.
Curve instability during sample purification	Air bubbles in the sample or buffer	Removal of air bubbles from samples or columns. Sample and buffer are degassed.
	Large temperature differences between the sample or buffer and the medium	Sample, buffer and medium are placed at the same temperature for purification.
No protein is eluted	Target proteins not expressed or expressed in low amounts	Optimize the expression of target proteins.
	Protein degradation or cleavage	Addition of appropriate amounts of protease inhibitors and protectants to the lysate. Purification at low temperature.
	Strong binding of target proteins	Increase biotin elution concentration.
The elute is not pure	Protein degradation or cleavage	Addition of appropriate amounts of protease inhibitors and protectants to the lysate. Purification at low temperature.
	Contaminant proteins interact with target proteins	Add a low concentration of reducing agent to the buffer and sample lysate. Add final concentration of 0.1% Triton X-100 to the buffer.
	Insufficient equilibration/wash operations	Increase the equilibrium liquid volume to ensure that the media is adequately equilibrated/washed, if the media is too dirty follow cleaning in place (part 3).

5. Related Products

Product	Cat. No.	Size
STarm Streptactin Beads 4FF	SA092005	5 ml
	SA092025	25 ml
	SA092100	100 ml
	SA092500	500 ml
	SA09201L	1 L
	SA09210L	10 L
PreCap STarm Streptactin	SA092C11	1×1 ml
	SA092C51	5×1 ml
	SA092C15	1×5 ml
	SA092C55	5×5 ml
	SA092CS	3×1 ml+1×5 ml