



# STarm Beads 4FF

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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 **Streptavidin ST** and **STarm**. Tag/ligand binding depends on the desired binding strength and application. The affinity of these two tags to **Streptavidin ST** and **STarm** is in the  $\mu\text{g/ml}$  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 Beads 4FF** is STarm Mutant coupled to highly cross-linked 4% agarose microspheres. Low concentrations (50  $\mu\text{mol/L}$ ) of D-Biotin in the sample do not affect the binding of the target protein to STarm 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.

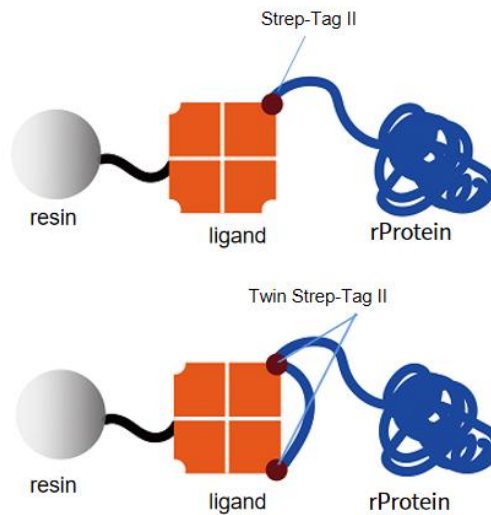


Figure 1. Schematic diagram of Strep-tag II and Twin Strep-tag II

Table 1. Characteristics of **STarm Beads 4FF**

Item	Description
Matrix	Highly cross-linked 4% agarose beads
Ligand	STarm Mutant
Capacity (/ml medium)	4 mg twin Strep-tag II fusion proteins
Particle Size ( $\mu\text{m}$ )	45-165 $\mu\text{m}$
Maximum Flow Rate	300 cm/h
Storage Buffer	1×PBS containing 20% ethanol
Storage Temperature	2-8°C





Table 2. Chemical compatibilities for STarm Beads 4FF

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 <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 0.1 M MgCl <sub>2</sub> 0.1 M CaCl <sub>2</sub>	1 hour

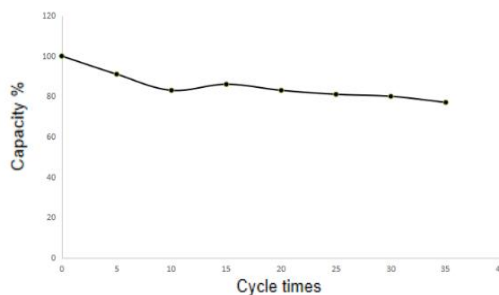


Figure 2. CIP Cleaning of STarm Beads 4FF

## 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 Packing Columns

STarm Beads 4FF is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. The method of packing the column is described below.

- 1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.
- 2) Close the column outlet leaving the net covered with packing buffer.
- 3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the





packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

- 4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **STarm Beads 4FF** is packed at a constant pressure of approximately 3bar(0.3MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400cm/h(10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Don't exceed 75% of the packing flow velocity in subsequent chromatographic procedures.
- 5) When the bed has stabilized, close the bottom outlet and stop the pump. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.
- 6) With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.
- 7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.
- 8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

### 2.3 Sample Purification

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

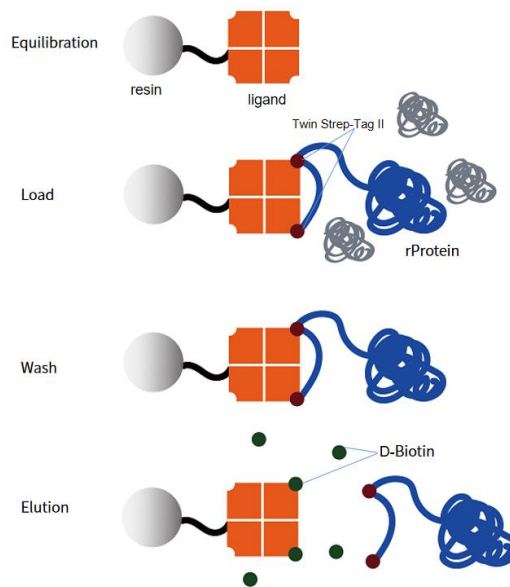


Figure 3. Schematic diagram of STarm Beads 4FF purification

- 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 Preservation

**STarm Beads 4FF** 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°C.





### 3. Cleaning-in-Place

In general, **STarm Beads 4FF** 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°C.

### 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 Beads 4FF	SA092005	5 ml
	SA092025	25 ml
	SA092100	100 ml
	SA092500	500 ml
	SA09201L	1 L
	SA09210L	10 L
PreCap STarm	SA092C11	1X1 ml
	SA092C51	5x1 ml
	SA092C15	1X5 ml
	SA092C55	5X5 ml
	SA092CS	3X1 ml+1X5 ml

