HiPur STarm 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 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 Beads 4FF** is STarm 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 Beads 4FF. The product can be regenerated with an equilibrium solution after use or cleaned with 10 mM NaOH.

HiPur STarm 4FF is a prepacked ready to use column. It is packed with 20 ml **STarm Beads 4FF**.The arrow on column label indicates the recommended flow direction. It can be adapted to all kinds of chromatography system, such as ÄKTA.

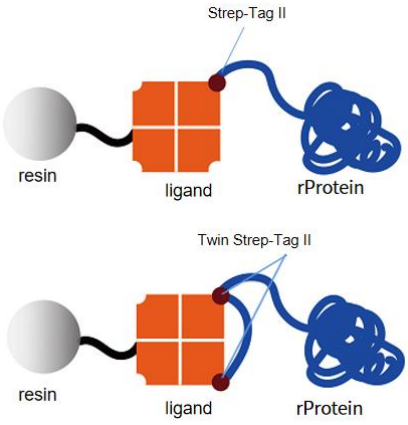

Figure 1. Schematic diagram of Strep-tag Ⅱ and Twin Strep -tag Ⅱ

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

Item	Description
Size	20 ml
Column size	1.6×10 cm
Matrix	Highly cross-linked 4% agarose beads
Ligand	STarm Mutant
Capacity （/ml medium）	4 mg Strep-tag II fusion proteins
Particle Size (μm)	45-165 μm
Maximum Flow Rate	300 cm/h
Storage Solution	1X PBS containing 20% ethanol
Storage Temperature	2-8℃

Table 2. Chemical compatibilities for **HiPur STarm 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 $(NH_4)_2SO_4$ 0.1 M $MgCl_2$ 0.1 M $CaCl_2$	1 hour

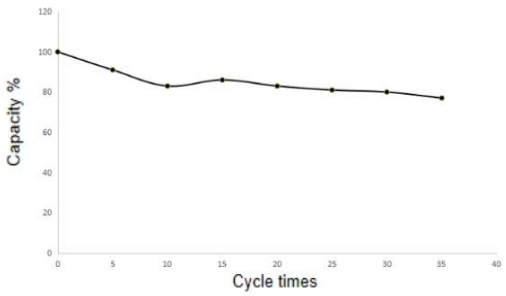

Figure 2. CIP Cleaning of STarm Beads 4FF

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 Sample Purification

HiPur STarm 4FF is a prepacked, ready to use column for purification. 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 elution buffer . For one-step elution, 5 column volumes are usually enough.

2.4 Analysis

Identify the fractions containing the target protein. Use UV absorbance, SDS-PAGE, or Western Blot.

3. Cleaning-in-Place

In general, **HiPur STarm 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℃.

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
HiSelect STarm 4FF	SA092C47	4.7 ml
HiPur STarm 4FF	SA092C20	20 ml