Instruction Manual

Strep-Tactin XT (Strep-tag Ⅱ) Agarose

Description

TargetMol Strep-Tactin XT Agarose utilizes a specialized protein coupling process to covalently conjugate Strep-Tactin XT protein onto the surface of highly cross-linked 4% agarose gel. It is a novel functionalized material specifically engineered for the separation and purification of Strep-tag II proteins, enabling highly efficient and rapid acquisition of high-purity purified proteins.

The Strep-Tactin XT Agarose utilizes the advanced Strep-Tactin/Strep-tag protein purification system, which mimics the streptavidin-biotin system to combine high specificity with mild elution conditions. Compared to other tags, Strep-tag II consists of a small 8-amino acid peptide tag (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys). The minimal size of this tag reduces potential interference with the structure or function of the target protein, eliminating the need for tag cleavage and preserving the structural and functional integrity of the fusion protein. The affinity of Strep-Tactin for Strep-tag II is increased nearly 100-fold compared to native streptavidin. Furthermore, the separation and purification conditions are gentle, allowing protein purification to occur under physiological conditions, which preserves protein biological activity and yields target proteins with a purity exceeding 99%.

Features

- **High Tolerance:** Tolerates 6 M Guanidine-HCl, 8 M Urea, 5 M NaCl, 50 mM DTT, 50 mM β-ME, 50 mM EDTA, 2% Tween-20, and 0.25 M Imidazole.
- **High Affinity & High Specificity:** Exhibits superior affinity toward both Strep-tag II and Twin Strep-tag II, effectively minimizing non-specific protein contamination.
- **Mild Elution Conditions:** Enables competitive elution utilizing D-biotin without the need for harsh denaturing or extreme conditions, thereby helping to maintain the native conformation and biological activity of the target protein.

Product Information

Property	Characteristics
Matrix	Highly cross-linked 4% agarose gel (4FF)
Particle Size	45–135 μm
Ligand	Recombinant Strep-Tactin XT protein
Gel Concentration	50% (v/v)
Binding Capacity	10 mg Twin Strep-tag II protein/mL gel
Pressure Tolerance Flow Rate	80–150 cm/h (0.3 MPa, 3 bar)

Applications

Suitable for the highly efficient purification of recombinant proteins carrying Strep-tag II or Twin Strep-tag II tags from a wide variety of expression systems, including baculovirus, mammalian cells, yeast, and bacteria.

Instructions

Reagents to be Prepared by the User

Reagent	Optional Formulation
Equilibration/Wash Buffer	0.15M NaCl, 20mM Na2HPO4, pH 7.2
Elution Buffer	Equilibration/Wash Buffer containing 1–5 mM D-Biotin
Regeneration Buffer	10mM NaOH

1. Sample Preparation

Prior to loading, it is recommended to centrifuge the sample and filter it through a 0.22 μm or 0.45 μm membrane to remove particulate impurities and improve protein purification efficiency.

2. Column Packing

1) Select an affinity chromatography column of appropriate dimensions. Install the bottom frit/gasket, add an appropriate volume of deionized water to rinse the column tube and frit, and close the bottom outlet.
2) Thoroughly mix the Strep-Tactin XT (Strep-tag II) Agarose to ensure a homogenous suspension. Pipette an appropriate volume of the slurry into the chromatography column, and open the bottom outlet to allow the storage solution to drain naturally.
3) Add an appropriate volume of deionized water to rinse the gel matrix. Once the liquid has completely drained, close the bottom outlet.
4) Equilibrate the packed chromatography column using Equilibration/Wash Buffer. If it is not for immediate use, add storage solution and store at 2–8°C.

3. Sample Purification

3.1 Batch/Incubation Method Purification

1) Based on the sample volume, transfer an appropriate amount of Strep-Tactin XT (Strep-tag II) Agarose into a centrifuge tube. Centrifuge at 1,000 rpm for 1 min, and discard the supernatant.
2) Add 5 gel volumes of Equilibration/Wash Buffer to wash the gel, centrifuge at 1,000 rpm for 1 min, discard the supernatant, and repeat at least twice.
3) Add the sample, and incubate on a rotary mixer at 2–8°C for 2–4 h or overnight.
4) Following incubation, centrifuge at 1,000 rpm for 1 min. Transfer the supernatant to a clean centrifuge tube (retain this as the flow-through fraction for subsequent analysis).
5) Add 5 gel volumes of Equilibration/Wash Buffer to wash the gel. Centrifuge at 1,000 rpm for 1 min, and discard the supernatant (avoid aspirating the gel matrix). Repeat this step 3–5 times; it is recommended to change the centrifuge tubes during this process.
6) Add 3–5 gel volumes of Elution Buffer to perform elution, and incubate at room temperature for 5–15 min. Centrifuge at 1,000 rpm for 1 min to collect the eluate. This step can be repeated 2–3 times.

3.2 Column Chromatography Method Purification

Note: Solution volumes are calculated based on the Column Volume (CV). For example, 5 column volumes (5 CV) correspond to 5 mL of solution for a 1 mL column specification, and 50 mL of solution for a 10 mL column specification.

1) Equilibrate the chromatography column with 5 column volumes of Equilibration/Wash Buffer to ensure the gel matrix is equilibrated with the target protein's buffer system, and repeat 2–3 times.
2) Load the sample onto the equilibrated chromatography column, incubate on a rotary mixer for 30–60 min, and collect the effluent. Sample loading can be repeated to increase binding efficiency.

3) Wash the column with 10–15 column volumes of Equilibration/Wash Buffer to remove non-specifically bound proteins, and collect the wash fractions.
4) Elute with 5–10 column volumes of Elution Buffer. Collect the eluate fractionally by column volume (collect one tube per 1 CV) and analyze each fraction separately to obtain the target protein at high purity and high concentration.

3.3 SDS-PAGE Analysis

Analyze the original sample and all components obtained during the purification process (flow-through, wash, and eluate) via SDS-PAGE to evaluate the purification efficiency.

4. Gel Regeneration and Storage

1) **Regeneration:** It is recommended to regenerate the gel after each use to remove bound D-biotin and ensure reproducible performance. The procedure is as follows:
   Wash sequentially with 5 column volumes of deionized water → treat with 10 column volumes of Regeneration Buffer → wash again with 5 column volumes of deionized water.
2) **Storage:** Regenerated gel should be suspended in an equal volume of storage solution and kept at 2–8°C to prevent microbial contamination.

Storage Conditions

Store at 4°C for 2 years.

Precautions

1. The gel must be maintained in the storage solution to prevent drying out.
2. Before withdrawing the agarose gel from the stock tube, shake thoroughly to ensure a homogenous suspension. Take care to avoid generating air bubbles during operation.
3. Operators may analyze the binding efficiency of the antibody and antigen to the gel by testing the supernatants collected during the antibody-binding and antigen-binding steps based on experimental needs.
4. In immunoprecipitation (IP) experiments, the binding affinity varies among different types of antibodies and antigens. If the buffer system provided in this kit fails to yield optimal experimental results, operators may screen and formulate customized buffers for optimization.
5. This product is intended solely for scientific research use by professional personnel. It must not be used for clinical diagnosis or treatment, food or drugs, and must not be stored in residential areas.
6. For your safety and health, please wear a lab coat and disposable gloves during operation.

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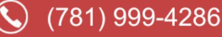

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