Strep-tag II Protein Purification Kit
PRODUCT DATA SHEET

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Description

The Strep-Tag system is a new protein purification system simulating the strep-biotin system. Compared with Strep-Tactin, the affinity of Strep-Tag II is at least 10 times stronger than that of strep-tavidin, and the separation and purification conditions are mild, so the separation and purification of proteins can be achieved under physiological conditions. In addition, compared with other tags, Strep-Tag II is a small tag of 8 amino acids (WSHPQFEK), which does not affect the structure and function of the protein after fusion due to the small tag, only about 1 kDa. These mild purification parameters preserve the biological activity of the protein and yield over 99% purity in just one step of extraction.

By using a special protein coupling technology, Abvigen Strep-Tactin magnetic beads covalently coupled Strep-Tactin protein to the surface of superparamagnetic magnetic beads, a new functional material is prepared for the efficient and rapid separation and purification of Strep-tag II protein. A platform for protein purification with both extraction speed, extraction quantity and purity was established.

For custom sizes, formulations or bulk quantities please contact our customer service department.

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Characteristics

Constituent	Magrose Strep-Tactin 5 mL
	Binding buffer 150 mL
	Desthiobiotin 2.65 mg
Particle size	30~150 μm
Ligand content	~6 mg Strep-Tactin /mL Gel
Fusion protein binding amount	~7 mg Strep-tag II protein /mL Gel
Concentration	10% (V/V)
Preservation solution	1×PBS, containing 0.1%Tween-20 and 0.1% Proclin 300

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Storage temperature	2°C ~ 30°C (long-term storage, recommended 2°C ~ 8°C)
Quality guarantee period	2 years
Binding/Washing Buffer	10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, containing 0.03% Proclin 300, pH 8.0
Elution Buffer	2.5 mM desthiobiotin in Binding Buffer
Regeneration Buffer	0.5 M NaOH or 1 mM HABA in Binding Buffer

Note: 1. The amount of protein binding of magnetic beads is related to the characteristics of the target protein and is only used as a reference value. 2. 1 mL magnetic bead suspension contains 100 μL magnetic beads.

Scope of application

It can be used to isolate and purify Strep-Tag II tagged proteins from any expression system, including baculoviruses, mammalian cells, yeast, and bacteria.

Operation process

The binding properties of target protein and magnetic beads will directly affect the purification efficiency of target protein, and the preparation of various buffers will also affect the recovery and purity of target protein to a certain extent. The following provides a more widely used Strep-Tag II protein purification process. Users can refer to the operation process, or design and optimize the protein purification process according to their own protein characteristics.

1. Buffer solution preparation

Binding/Washing Buffer: 10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

Elution Buffer: 2.5 mM desthiobiotin in Binding Buffer

2. Sample handling

This User Manual provides the following three sample handling methods:

(1) E. coli, yeast and other intracellular expression proteins: the expression cells were diluted with appropriate Binding Buffer and added with protease inhibitors (such as PMSF with a final concentration of 1 mM); The ice bath ultrasonic lysis of cells, which is a crude protein sample. If the sample is too thick, an appropriate amount of nuclease can be added to the crude sample as needed and placed on ice for 30 min to degrade nucleic acid. In addition, if the target protein content is low, it is recommended to centrifuge the crude protein sample.

(2) Extracellular expression protein: extracellular expression supernatant was taken and diluted with an equal amount of Binding Buffer to balance the crude protein sample.

(3) Intracellular expression of protein in animal cells: Appropriate amount of animal cells were taken, washed once with appropriate amount of PBS, and the supernatant was discarded; Resuspension with a Binding Buffer containing 1% (V/V) Triton X-100 or 1% (V/V) NP-40; Add protease inhibitors (e.g., PMSF with a final concentration of 1 mM); The crude protein sample is placed on ice for 10 minutes.

3. Magnetic bead pretreatment

In general, the amount of magnetic beads used is calculated by the user based on the target protein yield and magnetic bead load information. For example, Escherichia coli is used to express a target protein, and 1 g of wet weight bacteria is harvested from 250 mL fermentation solution. The target protein yield is estimated to be ~7 mg through pre-experiment, and the user needs to take 10 mL of 10% magnetic bead suspension for the purification of the target protein. The following is an example to explain in detail:

(1) The magnetic bead product was thoroughly mixed on the vortex mixer, and 10 mL magnetic bead suspension was taken by pipette into the centrifugal tube;

(2) Place the centrifugal tube on the magnetic separator, and remove the supernatant after the solution is clarified;

(3) Add 5~10 mL Binding Buffer/Washing Buffer into the centrifuge tube with magnetic beads, close the lid tightly, and swirl for 15 s to re-suspend the magnetic beads. Place the centrifugal tube on the magnetic separator, magnetic separation *, remove the supernatant, repeat washing 2 times.

(* Note: In the process of magnetic separation, in order to reduce the loss of magnetic beads during use, after the solution is clarified, close the centrifugal tube cover, keep the centrifugal tube still on the magnetic separator, and turn the magnetic separator and the centrifugal tube up and down several times, so that the clarified solution can be washed with the residual magnetic beads on the centrifugal tube cover, and let it stand for a moment, so that the solution can be re-clarified; Same as below.

4. The target protein binds to the magnetic bead

(1) The bacteria with 1 g wet weight were suspended with 10 mL Binding Buffer, and the crude protein samples were obtained after crushing and cracking;

(2) The crude protein sample was transferred to a centrifuge tube equipped with pre-treated magnetic beads, and the cover of the centrifuge tube was tightly closed;

(3) Place the centrifuge tube in a vortex mixer for 15 s, and then place it on a vertical mixer for 30 min at room temperature (if necessary, rotate and mix at a low temperature of 2-8°C for about 1 h to prevent degradation of the target protein);

(4) Place the centrifugal tube on the magnetic separator for magnetic separation, and remove the supernatant to the new centrifugal tube for subsequent detection. Remove the centrifugal tube from the magnetic separator for the next washing step.

5. Magnetic bead washing

(1) Add 5~10 mL of Washing Buffer into the magnetic bead in step 4, swirl it for 2 min, magnetically separate it, and remove the washing solution into a new centrifuge tube for sampling and testing;

(2) Continue to add 5~10 mL Washing Buffer to the above magnetic beads, swirl and mix for 2 min to re-suspend the magnetic beads and transfer the suspension of the magnetic beads to the new centrifuge tube to avoid contamination of the target proteins by non-specific adsorption proteins on the wall of the original centrifuge tube; Magnetic separation, remove the supernatant to the collection tube for sampling and testing;

6. Target protein elution

(1) Add 2-5 mL Elution Buffer (users can change the elution volume to adjust the target protein concentration as required) into the centrifuge tube, close the centrifuge tube cap, and then place the centrifuge tube on the vertical mixer, and vertically mix and elute for 10 min at room temperature; Magnetic separation, eluent collected into a new centrifuge tube, that is, the purified target protein sample;

(2) If necessary, the above steps can be repeated once to collect the sample into a new centrifuge tube to test whether the target protein is completely eluted.

7. Regeneration and preservation of magnetic beads

(1) NaOH regeneration: The magnetic beads after elution of the target protein are washed in the following order: 5~10 mL purified water was washed 3 times, 5~10 mL 0.5M NaOH was washed 3 times, 5~10 mL purified water was washed until neutral, and finally 10 mL preservation solution was added, and the magnetic beads were stored at 2~8°C.

(2) HABA regeneration: The magnetic beads eluted with the target protein can also be regenerated with HABA Buffer, adding 5~10 mL 1 mM HABA to wash the magnetic beads for 5 times, then washing the magnetic beads with Binding Buffer to the color of the magnetic beads, washing for 5 min each

time, and finally adding 10 mL preservation solution. The magnetic beads are stored in an environment of 2~8°C.

Optimization of protein purification process

The above operation flow applies to the purification of most Strep-Tag II label proteins. According to the difference in the binding properties of the target protein and the Strep-Tactin magnetic bead for protein purification, the user can optimize the purification flow from the following aspects to improve the recovery and purity of the target protein.

1. Reference methods to improve the recovery rate of target protein:

(1) Prolonging incubation time of protein solution and magnetic beads;

(2) Add appropriate protease inhibitors to prevent target protein degradation;

(3) Increase the amount of magnetic beads;

(4) Prolong the elution time of the target protein or increase the elution frequency;

2. Reference methods to improve the purity of the target protein:

(1) Add appropriate protease inhibitors in the purification process to prevent the degradation of the target protein;

(2) Extend the washing time and increase the number of washes;

Application example

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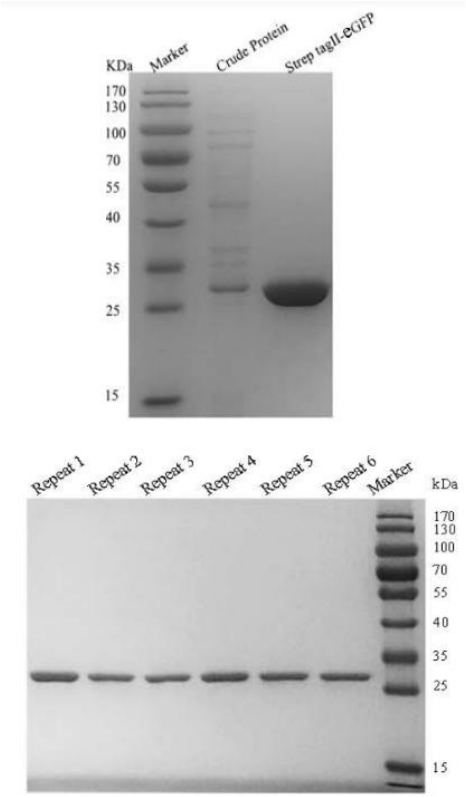

Strep-Tactin SDS-PAGE map of purified Strep-tagII-eGFP protein

The figure above shows that the Strep-tag II-eGFP target egg can be quickly and effectively purified under physiological conditions by using the Steptactin magnetic bead. With repeated use, the target egg self-purity can remain above 99%, and the high-purity target egg is convenient for further functional research.

Notes

(1) Please read this user manual carefully before using this product for the first time;

(2) Freezing, drying and high-speed centrifugation should be avoided during the use and preservation of magnetic beads;

(3) Before using this product, be sure to fully oscillate to keep the magnetic beads in uniform suspension;

(4) Please choose a good quality pipette suction head and centrifugal tube to avoid leakage of magnetic beads during the wall attachment or mixing process;

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(5) In the process of mixing the magnetic beads with the solution, if the solution is thick and cannot be re-suspended by turning the centrifugal tube, the pipette can be used to repeatedly suck or short-time vortex mixing to fully re-suspended the magnetic beads;

(6) The user can retain the supernatant removed by magnetic separation according to actual needs for sampling and testing in order to analyze the purification process and optimize the protein purification process;

(7) This product can be reused, when reused, it is recommended to purify the same protein, and when purifying different kinds of protein, it is recommended to use new magnetic beads to prevent cross-contamination;

(8) This product needs to be used with a magnetic separator;

(9) This product is for research use only.

Ordering Information

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