Strep-Tag II protein-purified magnetic beads (Beads Magrose Strep-Tactin)

Cat: M2350
Specification: 5 mL
Srorage: 2-8℃, valid for 2 years

Product content:

Strep-Tag system is a new protein purification system simulating streptavidin-biotin system. The affinity of Strep-Tactin is at least 10 times stronger than streptavidin, with mild separation and purification under physiological conditions. In addition, compared with other tag, Strep-Tag II is a small label of 8 amino acids (WSHPQFEK), due to the small label, only about 1 kDa, which does not affect the structure and function of the protein after fusion. These mild purification parameters preserve the biological activity of the protein and yield over 99% purity with only a one-step extraction. Beads Magrose Strep-Tactin magnetic beads use a special protein-coupling process to couple Strep-Tactin protein to the surface of superparamagnetic magnetic beads, prepare a new functionalized material for efficient and rapid separation and purification of Strep-tag II protein, and realize and build a protein purification platform with extraction speed, extraction amount and purity.

Product features:

product name	Beads Magrose Strep-Tactin
magnetic bead diameter	30~150 μm
content of ligand	~6 mg Strep-Tactin /mL Gel
Fusion protein binding capacity[1]	~7 mg Strep-tag II Protein / mL Gel
Suspension concentration	10% (v / v) magnetic bead suspension
preservative fluid	1 PBS (including 0.1% Tween-20 with 0.1% Proclin 300)
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 magnetic bead protein binding is related to the characteristics of the target protein, which is only used as a reference value here.

Scope of application:

Can be used for the isolation and purification of the Strep-Tag II tag-containing proteins from any expression system, including baculovirus, mammalian cells, yeast, and bacteria.

Operation procedures:

The binding performance of the target protein and the magnetic beads will directly affect the

purification efficiency of the target protein, and various buffer preparations will also affect the recovery and purity of the target protein to some extent. The following provides a 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 the characteristics of their own proteins.

1. Preparation of the buffer solution

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 processing

(1) Intracellular protein expression in *E.coli*, yeast, etc.: expressing cells were diluted with appropriate Binding Buffer and supplemented with protease inhibitor (such as PMSF with final concentration of 1 mM); ice bath ultrasound lysis, or 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 the nucleic acid. In addition, the crude protein sample is centrifugal if the target protein is low.

(2) Extracellular expression protein: the extracellular expression supernatant is taken and balanced with equal Binding Buffer dilution, which is the crude protein sample.

(3) Intracellular protein of animal cells: take appropriate amount of animal cells, wash once with appropriate amount of PBS and discard the supernatant; resuspend in appropriate amount of Binding Buffer containing 1% (v/v) Triton X-100 or 1% (v/ v) NP-40; add protease inhibitor (final PMSF);put on ice for 10 min, crude protein sample.

3.Magnetic beads pretreatment

Generally, the usage of magnetic beads is calculated by the user based on the target protein yield and magnetic bead load information. For example, *E.coli* was used to express a target protein, and 250 mL fermentation broth was harvested for 1 g of wet bacteria. The target protein yield was estimated to be ~7 mg through pre-experiment. The user needs to take 10 mL 10% of the magnetic bead suspension for the purification of the target protein. This is an example to detail:

(1) Mix the magnetic bead product on the vortex mixer thoroughly, and take 10 mL of magnetic bead suspension in the centrifuge tube.

(2) Place the centrifuge tube on the magnetic separator, and remove the supernatant after the solution is cleared.

(3) Add 5~10 mL Binding Buffer / Washing Buffer to the above, with the lid closed, and vortex for 15s to re-suspend the magnetic beads. The centrifuge tube was placed on a magnetic separator, magnetic separation *, the supernatant was removed, and the wash was repeated twice.

* Note: In the process of magnetic separation, in order to reduce the loss of the magnetic beads, cover the cover of the centrifuge tube and keep the centrifuge tube on the magnetic separator, flip the handheld magnetic separator and the centrifugal tube several times, make the clarified solution wash the residual magnetic beads on the centrifuge tube, and stand for a while to clarify the solution again; below.

4.Binding of target protein to magnetic beads

(1) After 10 mL Binding Buffer 1 g of wet bacteria were resuspended, crushing and lysis, the crude protein sample was used.

(2) Transfer the crude protein sample to a centrifuge tube with pretreated magnetic beads and cover the centrifuge tube tightly.

(3) Put the centrifuge tube in the vortex mixer for 15s, and then put it on the vertical mixing instrument, mix vertically at room temperature for 30 min (if necessary, rotate and mix at a low temperature of 2~8℃ for about 1 h to prevent degradation of target protein).

(4) The centrifuge tube was placed on a magnetic separator for magnetic separation, and the supernatant was removed into a new centrifuge tube for subsequent testing. Remove the centrifuge tube from the magnetic separator for the next washing step.

5. Magnetic beads washing

(1) Add 5~10 mL Washing Buffer to the magnetic beads in step 4, mix the vortex for 2 min, magnetic separation, and remove the cleaning liquid to a new centrifuge tube for sampling and detection;

(2) Continue to add 5~10 mL Washing Buffer to the above magnetic beads and mix the vortex for 2 min to re-suspend the magnetic beads, transfer the magnetic bead suspension to a new centrifuge tube to avoid non-specific adsorption protein contamination from the original centrifugal tube wall; magnetic separation, remove the supernatant to the collection tube for sampling and detection;

6.Target protein elution

(1) Add 2~5 mL Elution Buffer (the user can change the elution volume and adjust the target protein concentration as needed) to the centrifuge tube, cover the centrifuge tube tightly, and then place the centrifuge tube on the vertical mixing instrument, mix and elute vertically at room temperature for 10 min; magnetic separation, and collect the eluate into a new centrifuge tube, which is the purified target protein sample;

(2) If required, the above procedure can be repeated once and the sample is collected into a new centrifuge tube to detect whether the target protein is completely eluted.

7.Magnetic beads regeneration and preservation

(1) NaOH Regeneration: the magnetic beads after eluting the target protein were washed in the following order: 5~10 mL of purified water 3 times, 5 ~ 10 mL 0.5 M NaOH 3 times, 5 ~ 10 mL of purified water to neutral, 10 mL of storage solution was added, and the magnetic beads were placed for 2~8℃ environment.

(2) HABA regeneration: the magnetic beads eluting the target protein with desulfurized biotin can also be regenerated with HABA buffer. After adding 5~10 mL 1mM HABA, the magnetic beads were washed 5 times, then the magnetic beads were washed with Binding Buffer to the color of the beads themselves for 5 min, finally adding 10 mL of storage solution, and the magnetic beads were stored for 2~8°C environment.

Optimization of the protein purification process

The above operation process is suitable for the purification of most Strep-Tag II tag proteins. According to the different binding performance of the target protein and the Strep-Tactin protein purification magnetic beads, users can optimize the purification process from the following aspects to improve the recovery and purity of the target protein.

Reference methods for improve the recovery of target proteins:

1. Extend the incubation time of protein solution and magnetic beads.
2. Add appropriate protease inhibitors to prevent the degradation of the target proteins.
3. Increase the amount of magnetic beads.
4. Extend the elution of the target protein or increase the number of elution.

Reference methods for improving the purity of the target protein:

1. Appropriate protease inhibitors were added during the purification process to prevent the degradation of the target protein.
2. Extend the washing time, increase the washing times.

Note:

1. Please read the user manual before first using the product.
2. Frozen, drying and high-speed centrifugation should be avoided during the use and storage of magnetic beads.
3. Before using this product, be sure to keep the magnetic beads in uniform suspension state.
4. Please choose a good quality pipette suction head and centrifuge tube to avoid the loss of magnetic beads caused by leakage in the wall or mixing process.
5. In the process of mixing the magnetic beads and the solution, if the solution is thick and cannot be resuspended by the flipped centrifuge tube, the magnetic beads can be fully resuspended by the pipette repeatedly or the short-term vortex mixture.
6. According to the actual needs, users can keep the supernatant removed by magnetic separation for sampling and testing, so as 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 purify different kinds of protein, it is recommended to use new magnetic beads to prevent cross-contamination.
8. This product should be used together with the magnetic separator.
9. This product is intended for study use only.