Strep-tag II Protein Purification Kit

Catalog No.: BRC0022

Product Introduction

The Strep-Tag system is a novel protein purification platform that mimics the streptavidin-biotin interaction. Strep-Tactin exhibits at least 10-fold higher affinity for Strep-Tag II compared to streptavidin, enabling purification under gentle, physiological conditions that preserve protein bioactivity. The Strep-Tag II tag is a small 8-amino-acid sequence (WSHPQFEK, ~1 kDa) that minimally affects fusion protein structure and function, yielding >99% purity in a single step.

EnkiLife Strep-Tactin magnetic beads utilize a proprietary protein coupling technology to covalently immobilize Strep-Tactin onto superparamagnetic beads. This creates a high-performance purification platform that achieves an optimal balance of speed, capacity, and purity.

Product Information

Parameter	Specification
Strep-Tactin Beads	5mL
Binding Buffer	150mL
Desthiobiotin	2.65 mg
Bead Size	30–150 μm
Ligand Density	~6 mg Strep-Tactin/mL gel
Binding Capacity	~7 mg Strep-Tag II protein/mL gel*
Suspension Concentration	10% (V/V) bead suspension
Storage Buffer	1×PBS containing 0.1% Tween-20, 0.1% Proclin 300
Storage Temperature	2–30°C (For long-term storage, 2–8°C recommended)
Shelf Life	2 years
Binding/Washing Buffer	10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 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

*Binding capacity varies depending on target protein characteristics; value provided is for reference only.

Scope of Application

Suitable for purification of Strep-Tag II-tagged proteins from any expression system, including baculovirus-infected insect cells, mammalian cells, yeast, and bacteria.

Operating Protocol

The binding efficiency of target protein to beads directly affects purification yield. Buffer composition also influences protein recovery and purity. The following protocol is a widely applicable

workflow. Users may optimize conditions based on their specific protein characteristics.

Buffer 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

Sample Preparation

A. Intracellular Expression (E. coli, yeast):
1) Resuspend cell pellet in Binding Buffer + protease inhibitors (e.g., 1 mM PMSF final).
2) Lyse by sonication on ice.
3) If viscous, add DNase and incubate on ice for 30 min.
4) Centrifuge if necessary for clarification.

B. Secreted Expression: Collect culture supernatant and dilute 1:1 with Binding Buffer.
C. Mammalian Intracellular Expression:
1) Harvest cells, wash once with PBS, discard supernatant.
2) Resuspend in Binding Buffer containing 1% (V/V) Triton X-100 or NP-40 + protease inhibitors.
3) Incubate on ice for 10 min.

Bead Pretreatment

Calculate bead volume based on expected protein yield and bead capacity.
Example: 250 mL E. coli culture → 1 g wet cell weight → ~7 mg target protein → Use 10 mL of 10% bead suspension.

1. Vortex bead stock vigorously to resuspend.
2. Transfer 10 mL bead suspension to a centrifuge tube.
3. Place on magnetic separator until solution clears. Remove supernatant.
4. Add 5–10 mL Binding Buffer, vortex 15 sec, perform magnetic separation, and remove supernatant. Repeat wash twice.

Pro Tip: After solution clears, keep tube on magnet, close lid, invert magnet+tube several times to rinse beads adhering to the lid. Let settle and remove supernatant. This minimizes bead loss.

Target Protein Binding

1. Resuspend 1 g wet cell pellet in 10 mL Binding Buffer, lyse, and clarify to obtain crude sample.
2. Transfer crude sample to pretreated beads. Cap securely.
3. Vortex 15 sec, then rotate 30 min at room temperature (or 1 h at 2–8°C to prevent degradation).
4. Magnetically separate and transfer supernatant to a new tube for analysis.
5. Proceed to washing.

Bead Washing

1. Add 5–10 mL Washing Buffer, vortex 2 min, magnetically separate, and collect wash solution for sampling.
2. Add 5–10 mL Washing Buffer, resuspend, and transfer bead suspension to a NEW tube to avoid contamination from non-specifically adsorbed proteins on the tube wall. Magnetically separate and discard supernatant.

Protein Elution

1. Add 2–5 mL Elution Buffer to beads (adjust volume for desired concentration). Cap securely.
2. Rotate 10 min at room temperature for vertical mixing.
3. Magnetically separate and collect eluate—this is your purified protein.

4. Optional: Repeat elution once to verify complete recovery.

Bead Regeneration and Storage

Method 1: NaOH Regeneration

1. Wash sequentially:
    - 5–10 mL purified water: 3 times
    - 5–10 mL 0.5 M NaOH: 3 times
    - 5–10 mL purified water: until neutral pH
2. Add 10 mL Storage Buffer and store at 2–8°C.

Method 2: HABA Regeneration (for desthiobiotin-eluted beads)

1. Wash 5 times with 5–10 mL 1 mM HABA (5 min each wash).
2. Wash with Binding Buffer until beads return to original color.
3. Add 10 mL Storage Buffer and store at 2–8°C.

Protocol Optimization

To Improve Recovery:

1. Extend protein-bead incubation time.
2. Add protease inhibitors to prevent degradation.
3. Increase bead volume.
4. Extend elution time or increase elution cycles.

To Improve Purity:

1. Add protease inhibitors throughout purification.
2. Extend washing time and increase wash cycles.
3. Use gradient desthiobiotin concentration for elution.

Important Notes

1. Read this manual thoroughly before first use.
2. Avoid freezing, drying, or high-speed centrifugation of beads.
3. Ensure beads are fully resuspended before each use by vigorous vortexing.
4. Use high-quality, low-protein-binding consumables to prevent bead loss.
5. For viscous solutions, use pipette mixing or brief vortexing if inversion is insufficient.
6. Save all supernatants and washes for process analysis.
7. Beads can be reused for the same protein. Use fresh beads for different proteins to avoid cross-contamination.
8. A magnetic separator is required for operation (see compatible models below).
9. For Research Use Only (RUO)—not for diagnostic or therapeutic applications.