Streptavidin Magnetic Beads

Product Description

The BioLinkedin[™] Streptavidin Magnetic Beads provide a fast and convenient method for manual or automated immunoprecipitation, protein interaction studies, DNA-protein pulldowns, and purification of biotin-labeled proteins and nucleic acids. Biotinylated molecules are simply added to the streptavidin-coated magnetic beads for binding. The beads are removed from the solution manually using a magnetic stand or by automation using an instrument.

Product Features

Composition	Streptavidin covalently coupled to	
	magnetic bead surface	
Magnetization	Superparamagnetic	
Particle size	200 nm	
Concentration	10 mg/mL	
Binding Capacity	\ge 0.075 mg biotinylated rabbit IgG/	
	mg of beads	
Application	IP, CoIP	
Storage Condition	Store at 4°C for 2 years.	

Protocol

1. Preparation of Magnetic Beads

1.1 Resuspend the Magnetic Beads in the vial (tilt and rotate for 2 minutes or gently pipette for 10 times).

1.2 Transfer 50µL of Streptavidin Magnetic Beads into a 1.5mL tube (Transfer amount may be adjusted as required).

1.3 Add 1mL of binding/wash buffer to the beads and gently pipette to mix. Place the tube into a magnetic stand to collect the beads against the side of the tube (Herein after referred to as magnetic separation). Remove and discard the supernatant. Repeat wash twice.

2. Immunoprecipitation

Note: This protocol is a general guideline for immunoprecipitation and will require optimization for each application.

2.1 Combine the antigen sample with $10\mu g$ of biotinylated antibody. Incubate 1-2 hours at room temperature or overnight at 4°C with mixing.

Note: Dilute each sample to a minimum volume of 300µL with cell lysis buffer or Binding/Wash Buffer.

2.2 Add the antigen sample/biotinylated antibody mixture to a 1.5mL microcentrifuge tube containing pre-washed magnetic beads and incubate at room temperature for 1 h with mixing.

2.3 Collect the beads with a magnetic stand and remove and collect the supernatant for analysis.

2.4 Add 300μ L of Binding/Wash Buffer to the tube and gently mix. Collect the beads and then discard the supernatant. Repeat this wash twice.

3. Elution

Elution Buffer Recovery of Antigen:

 $3.1 \text{ Add } 100 \mu \text{L}$ of Elution Buffer to the tube.

3.2 Incubate the tube at room temperature with mixing for 5 minutes.

3.3 Magnetically separate the beads and collect the supernatant containing target antigen.

Note: If a low pH elution buffer is selected for elution, streptavidin leaching might occur. Low pH elution buffers are effective for most antibody-antigen interactions; however, to ensure efficient release of target antigen from the antibody, pre-rinse the beads with 300µL of 0.1% Tween-20 Detergent in water (no buffering capacity) before adding Low pH Elution Buffer.

SDS-PAGE Reducing Sample Buffer Recovery of Antigen: 3.4 Add 100 μ L of SDS-PAGE reducing sample buffer to the tube and heat the samples at 96-100°C in a heating block for 5 minutes.

3.5 Magnetically separate the beads and collect the supernatant containing target antigen.

Note: If SDS-PAGE buffer is selected for elution, the eluate will contain streptavidin monomers and dimers and biotinylated antibody along with target antigen.

Troubleshooting

Problem	Possible Cause	Solution
Low protein recovery	Proteolysis of sample	Add protease inhibitors
	Not enough magnetic beads used for capture	Increase the amount of magnetic beads used for capture
	Insufficient amount of target protein in the sample	Increase amount of antigen sample
Protein does not elute	Elution conditions were too mild	Increase incubation time with elution buffer or use more stringent elution buffer
Multiple, nonspecific bands appear in eluted sample	Nonspecific protein binding to the magnetic beads	Add 50-200mM NaCl to the Binding/Wash and Elution Buffers
Recovered protein is inactive	Elution conditions were too stringent	Use a milder elution buffer
Magnetic beads aggregate	Magnetic beads were frozen or centrifuged The buffer was incompatible with magnetic beads	Handle the beads as directed in the instructions