Protein Purification Gel, 45-135 µm-Streptavidin
PRODUCT DATA SHEET

Protein Purification Gel, 45-135 µm-Streptavidin

Cat No: AG-4500-Streptavidin

Description

Streptavidin gels, also known as Streptavidin gels or SA gels, are composed of highly cross-linked 4% agarose gels that covalently bind large amounts of high-quality streptavidin to the matrix. The interaction between biotin and streptavidin ligand is used to purify biotin or biotinylated proteins, antibodies and other substances. The affinity between streptavidin and biotin is very strong and needs to be eluted under denaturation conditions. Streptavidin has a relatively weak affinity for iminobiotin and can be combined at pH 9.5-11.0 and eluted at pH 4.0, so it does not need to use denaturants, so it can better maintain the activity of avidin conjugate.

Streptavidin gels are mainly used for the separation and purification of biotin-labeled nucleic acids, antibodies, proteins or related complexes, and can also be used for immunoprecipitation (IP), cell sorting, DNA-protein interaction studies, etc.

Characteristics

Matrix	Highly crosslinked 4% agarose gel (4FF)
Ligand	Recombinant Streptavidin protein
Particle size	45~135 µm
Concentration	The gel volume accounts for 50% of the suspension volume
Pressure flow rate	80 ~ 150 cm/h (0.3 MPa, 3 bar)
Binding ability	≥ 10 mg biotinized IgG/mL gel ≥ 15 mg biotinized BSA/mL gel
Scope of application	① Separation and purification: binding biotinized proteins, antibodies, etc.; ② Molecular interaction: IP, Co-IP, RNA Pulldown, etc.
Shelf life	Stable storage at 2 ~ 8°C for two years

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Operation process

(1) Biotinylation molecular purification process

1. Prepare before use

1.1 Buffer:

Purification of biotin or biotinylated molecules

Buffer	Formula
Balance/washing solution	0.15 M NaCl, 20 mM Na_2HPO_4, pH 7.2
Eluate	8 M guanidine hydrochloride, pH 1.5

Note: Guanidine hydrochloride elution and SDS will precipitate. Do not use guanidine hydrochloride elution for SDS-PAGE running. Use 0.1%SDS and boil for 5 min.

Purification of iminobiotin label molecules

Buffer	Formula
Balance/washing solution	0.5 M NaCl, 50 mM ammonium carbonate, pH 10.0
Eluate	0.5 M NaCl, 50 mM ammonium carbonate, pH 4.0

1.2 Vortex oscillator, rotary mixer, pipette, gun head and centrifugal tube

2. Sample preparation

Before loading, ensure that the sample solution has the appropriate ionic strength and pH, dilute the serum sample, ascites, or cell culture solution with **Balance/washing solution**, or place the sample on **Balance/washing solution**. It is recommended that the samples be centrifuged and filtered with 0.22 μm or 0.45 μm filter membrane before loading to reduce impurities and improve the efficiency of protein purification.

3. Gel filling

1) Take the appropriate size of the affinity chromatographic column, put it into the gasket, add an appropriate amount of deionized water washing column tube and gasket, and close the lower outlet.

2) Mix **streptavidin gel** evenly, use a pipette to absorb appropriate amount of slurry and add it to the chromatographic column (the actual volume of gel accounts for half of the suspension), and open the lower outlet to discharge the protective liquid.

3) Add appropriate amount of deionized water to rinse the gel, and close the lower outlet when the liquid in the column flows out.

4) The loaded chromatographic column is added to **Balance/washing solution** for balance, and the protective liquid is added when not used for the time being, and stored at 2 ~ 8°C.

4. Sample purification

4.1 Purification by incubation

1) According to the purified sample size, appropriate amount of **streptavidin gel** was added into the centrifuge tube, centrifuged at 1000 rpm for 1 min, and the supernatant was removed;

2) Add 5 times the volume of gel **Balance/washing solution** into the centrifuge tube to clean the gel, centrifuge at 1000 rpm for 1 min, remove the supernatant, repeat more than 2 times.

3) The samples were added and incubated at 2~8°C for 2 ~ 4 h or overnight.

4) After incubation, centrifuge at 1000 rpm for 1 min, absorb the supernatant to the new centrifuge tube, and retain the supernatant as a flow for electrophoresis identification.

5) Clean the gel with a **Balance/washing solution** of 5 times the gel volume, centrifuge at 1000 rpm for 1 min, remove the supernatant (be careful not to inhale the gel), repeat 3 to 5 times, and it is recommended to replace a new centrifuge tube in the middle.

6) Add 3 ~ 5 times the gel volume of **eluent** for elution, incubate at room temperature for 5~15 min, centrifuge at 1000 rpm for 1 min to collect eluent, can be repeated 2 ~ 3 times.

4.2 Purification by chromatographic column

The amount of solution is calculated by the column volume (for example, 5 times the column volume, 1 mL specification corresponds to 5 mL solution, 10 mL specification corresponds to 50 mL solution).

1) The loaded **streptavitin gel** chromatography column is balanced with 5 times the column volume **Balance/washing solution**, so that the gel is in the same buffer liquid system as the target molecule, and repeated 2 ~ 3 times.

2) The sample was added to the balanced chromatographic column and incubated in the rotary mixing apparatus for 30 ~ 60 min, then the effluent was collected, and the sample could be repeated to increase the binding efficiency.

3) Use 10 ~ 15 times the column volume of **Balance/washing solution** for cleaning, remove impurities unless specific adsorption, collect detergent.

4) Use **eluent** of 5 ~ 10 times column volume to eluate, collect in sections, collect one tube for each column volume, and test separately, which can ensure that all the combined target molecules are elated, and can get high purity and high concentration of molecules.

5. Gel wash

Streptavidin gel can be reused without regeneration, but with the precipitation of some denaturating substances and the aggregation of proteins, the flow rate and binding load are often reduced, which seriously affects the performance of the column. At this time, the gel needs to be cleaned.

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Remove some precipitated or denatured material

Clean with 2 times the column volume of 0.1 M NaOH or 6 M guanidine hydrochloride or 8 M urea, then immediately clean with 5 times the column volume of PBS (pH 7.4).

Remove some non-specific adsorbent substances caused by hydrophobic adsorption

Clean with 70% ethanol of 3 ~ 4 times column volume or 1% Triton X-100 of 2 times column volume, then immediately clean with 5 times column volume of PBS (pH 7.4).

(2) Biotinylated antibody immunoprecipitation operation process

Note: Prepare your own buffer or purchase our IP kit directly.

Preparation of immune complex

Note: The amount and incubation time required for the sample are dependent on each specific antibody antigen system and may need to be optimized for maximum yield.

The following protocol is for 2 ~ 10 µg biotinylated antibodies, which can be scaled up as required.

1. In a centrifuge tube, combine the cell lysate of each sample with 2 ~ 10 µg biotinylated antibody. The recommended total protein amount for each immunoprecipitation reaction is 500 ~ 1500 µg.
2. Dilute the antibody and prepared sample to 300 ~ 500 µL from IP Lysis/Wash Buffer.
3. Incubate at room temperature for 1 ~ 2 h, or 4°C for 2 ~ 4 h to form immune complexes.

Immunoprecipitation:

Note: To ensure an even distribution of the gel, mix the gel in the bottle by repeatedly reversing or slightly swirling it before use.

1. Add 20 to 50 µL of Streptavidin gel into 1.5 mL centrifuge tube.
2. Add 500 µL of pre-cooled PBS to the gel and mix gently.
3. Put the centrifuge tube into the centrifuge at 1000 rpm for 5 min to collect the gel to the bottom of the centrifuge tube and remove the supernatant.
4. Add 200 ~ 500 µL IP Lysis/Wash Buffer to the centrifuge tube. Reverse the centrifugal tube several times or mix with a slight vortex for 1 min. Put the centrifuge tube into the centrifuge at 1000 rpm, collect the gel to the bottom of the centrifuge tube for 5 min, and remove the supernatant.
5. Add the antigen sample/antibody mixture into the centrifuge tube with gel and keep it mixed for incubation at room temperature for 1 ~ 2 h, or 4°C for 2 ~ 4 h.
6. Centrifuge 1000 rpm, collect gel for 5 min, remove unbound sample, and store for analysis.
7. Add 1000 µL IP Lysis/Wash Buffer to the centrifuge tube and mix gently for 5 ~ 10 min. Collect the gel and discard the supernatant. Repeat twice.

8. **Denaturing elution:** Add 80 ~ 100 µL SDS-PAGE Sample Loading Buffer (1×) to the centrifuge tube and heat the sample in 100°C water bath or metal bath for 10 min. The gel is separated by centrifugation to retain the supernatant containing the target antigen.

Note: The following elution methods can also be used to maintain protein activity.

Low pH Elution: Add 100 µL Elution Buffer to centrifuge tube. Keep mixed and incubate centrifuge tube at room temperature for 5 ~ 10 min. Magnetic beads are magnetically separated to retain the supernatant containing the target antigen. Low pH is neutralized by adding 20 µL Neutralization buffers per 100 µL of wash out solution.

Notes

1. Before performing the experiment, please read this operation manual carefully.
2. The ability of SA to bind to biotinylated molecules in the experiment is different, and the binding is also affected by Buffer, so **the operation details can be optimized or the buffer can be screened and prepared for the experiment**.
3. The gel should be fully oscillated and evenly before use. The gel should be kept in storage solution to prevent drying.
4. This product is for scientific research only.

Ordering Information

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