Anti-V5（GKPIPNPLLGLDST）Affinity Agarose

Cat. No: EA-IP-010 **Size: 2 mL**

Note: *Do not centrifuge and use after mixing gently.*

Performance metrics

Scope of application	Affinity purification and immuno(co)precipitation of V5 tag fusion proteins. The V5 tag can be located at the N-terminus, C-terminus or in the middle of the protein, such as N-terminal V5 fusion protein (V5‑Protein), C-terminal V5 fusion protein (Protein-V5) and Met-modified N-terminal V5 fusion protein (Met-V5‑ Protein). Suitable for secreted proteins.
Antibody properties	Rabbit Polyclonal Antibody: IgG Subtype 2a.
Gel properties	Agarose gel granules, average size 100~200 μm.
Binding capacity	1mL Sepharose 4B agarose particles, covalently conjugated to 6mg mouse-derived IgG. 1mL affinity gel can purify or precipitate at least 1.2mg V5 fusion protein.
Components	1mL Anti-V5 affinity gel in 1mL PBS with preservative and 50% glycerol.

Matters Needing Attention

1. This product is only for scientific research by professionals and may not be used for clinical diagnosis or treatment.
2. For your safety and health, please wear a lab coat and disposable gloves.
3. This product provides affinity magnetic beads in the form of suspension. Gently re-suspend the magnetic bead suspension before use, and then use it as needed.
4. Do not centrifuge, freeze or dry the magnetic beads, do not sonicate the magnetic beads, and do not allow acid treatment of the magnetic beads for more than 10 minutes.
5. When mixing the magnetic beads, please use methods such as gentle pipetting with a pipette, gentle vortexing, inversion, and shaker mixing. Do not use sonication and other methods.
6. The relevant reagents used must be prepared by the laboratory.

Method of Application

1. Sample Preparation of Target Proteins

1) Sample processing serum and recombinant proteins

Collect serum or culture medium supernatant and detect the target protein concentration. If the target protein concentration is high, it is recommended to dilute it with 1×PBS to a final protein concentration of 10~100μg/mL for subsequent experiments.

2) Sample processing of target protein for intracellular expression

a. Blow off in case of adherent cells or take suspension cells from the cell culture flask and transfer them to a centrifuge tube, centrifuge at 1000 rpm for 5 min, and discard the supernatant。

b. Re-suspend cells in 1× PBS pre-cooled at 4 °C, centrifuge at 1,000 rpm for 3 min, and discard the supernatant. Repeat once.

c. Add the corresponding volume of cell lysate according to the amount of cells, and place on ice for 10~20 min after repeated pipetting.

Note: Generally, 1mL of cell lysis solution can process about 0.5~1×10^7 cells. To avoid degradation of your target protein, you can add protease inhibitors.

d. Use a sonicator to treat the cell lysate until the cell lysate is transparent and no longer viscous. After placing on ice for 30 minutes, centrifuge at 12,000 rpm and 4°C for 10 minutes. Take the supernatant for subsequent experiments.

2. Column Installation and Incubation

1) Anti-V5 Affinity Agarose preparation

a. Gently re-suspend the Anti-V5 Affinity Agarose, mix evenly, and take 40 μL gel suspension (containing approximately 20 μL gel) into a centrifuge tube.

b. Add 10 times the gel volume (about 200 μL) of 1×PBS to gently re-suspend and wash the gel, centrifuge at 5 000 rpm for 30 seconds, discard the supernatant, and repeat this step three times.

Note: For multiple samples, the gel can be re-suspended and divided into several reaction tubes for separate reactions.

2) Binding of target protein to Anti-V5 Affinity Agarose

a. Incubation: Add 200 μL of the prepared sample to the washed gel, and incubate on a shaker at room temperature for 2 hours. It can also be incubated at 4°C overnight or longer.

b. Washing: After incubation, centrifuge at 5000rpm for 30 seconds and discard the supernatant. Add 200 μL 1×PBST, mix gently, wash the gel, centrifuge at 5000 rpm for 30 seconds, discard the supernatant, and repeat this step 4 times.

3) Target protein elution

This instruction manual provides the following two target protein elution schemes. Please choose different target protein elution methods according to the needs of later detection.

Denaturing elution method

This method is only suitable for SDS-PAGE detection.

a. Add 16 μL 1×PBS and 4 μL 5× loading buffer, boil the sample for 5 minutes, cool it down to room temperature and centrifuge.

b. Take the supernatant and run SDS-PAGE in preparation for subsequent Western Blot detection.

Acid elution method

Acidic elution method has low cost, short operational time, generally does not cause protein denaturation, and facilitates subsequent analysis and detection of proteins.

a. Add pre-cooled acidic eluent pH 3.0, 10 times of the gel volume (approximately 200 μL), to the above precipitate, suspend the affinity gel, and incubate at room temperature for 5 minutes.

Note: An acidic environment will shorten the service life of the gel. The contact time between the gel and the acidic eluent should be shortened as much as possible. It is recommended not to exceed 10 minutes.

b. After the incubation, centrifuge at 5000 rpm for 30 seconds at 4°C, transfer the supernatant to a new centrifuge tube, and immediately add 1/10 volume of neutralizing solution pH 8.0 and mix well. The supernatant is the eluted V5-tagged protein.

c. Process and store proteins according to subsequent experimental needs.

Background

Anti-V5 (GKPIPNPLLGLDST) Affinity Agarose is made of high-quality V5 tag antibodies covalently conjugated to agarose gel. It has the characteristics of high loading capacity, high specificity, stable properties, and can be used repeatedly. It can be used for Immunoprecipitation-related experiments such as affinity purification, immunoprecipitation (IP), co-immunoprecipitation (Co-IP), etc.

Storage

-20°C for 12 months.