Protein A/G Agarose Beads

（AGA001）

Product Overview

Native Protein A is a cell wall surface protein identified in *Staphylococcus aureus*, and native Protein G is a cell surface protein isolated from *Streptococcus* species. These two proteins share similar functions, primarily interacting with the Fc region of immunoglobulin G (IgG) and binding to IgG from most mammalian species. Protein A/G Agarose Beads are prepared by the covalent conjugation of Protein A and Protein G to agarose beads. They can be used for protein function-related research via Immunoprecipitation (IP) or Co-Immunoprecipitation (CoIP), or packed directly into chromatography columns for the purification of monoclonal or polyclonal antibodies.

Components

Product Name	Cat. No.	Volume
Protein A/G Agarose Beads	AGA001-01	50 μL
	AGA001-02	250 μL

Storage Conditions

Store at 4℃ for a shelf life of 1 year, or at -20℃ for a shelf life of 2 years. Transport with ice packs.

Notes

1. For your health and safety, wear a lab coat and disposable gloves during operation. This product is for scientific research use only.
2. For long-term storage, store the product at -20℃. Avoid centrifugation, desiccation and repeated freeze-thaw cycles.
3. The storage buffer of this product is PBS containing 20% ethanol and 0.03% NaN_3.

Experimental Procedures

1. Cell Harvesting:

1) **Adherent cells**: Take adherent cells from a 10 cm cell culture dish, aspirate the culture medium, wash the cells twice with PBS, and add 1 mL of cell lysis buffer (e.g., regular IP lysis buffer or mild RIPA lysis buffer, supplemented with protease inhibitor cocktail before use) to lyse the cells.
2) **Suspension cells**: Collect cells by centrifugation, wash once with PBS, and lyse the cells following the lysis method for adherent cells.
3) **Animal and plant tissue samples**: Lyse the samples at the same lysis buffer-to-sample ratio used for adherent cells;

plant tissues require pre-grinding in liquid nitrogen before lysis.

Note： Use an appropriate volume of lysis buffer for different cell quantities. If the concentration of the lysed protein sample is too high, dilute it properly with lysis buffer or PBS; if the concentration is too low, reduce the volume of lysis buffer appropriately during lysis. A total protein concentration of 1.0~2.0 mg/mL in the lysis buffer is generally optimal. If the target protein concentration is low, the total protein load can be increased.

2. Pre-clearing (Optional)：

1) Take 0.5~1 mL of cell or tissue lysate containing approximately 1.0~2.0 mg of protein, add about 1 μg of normal IgG from the same species as the IgG used for subsequent immunoprecipitation and 20 μL of fully resuspended Protein A/G Agarose Beads, and mix by rotation at 4℃ for 1~2 hours.
2) Centrifuge at 2500 rpm (approximately 1000 g) for 5 minutes, and collect the supernatant for subsequent immunoprecipitation experiments.

Note： Normal IgG of the same species refers to non-immune IgG from the host of the primary antibody used in subsequent immunoprecipitation. This step pre-clears proteins in the sample that non-specifically bind to Protein A/G Agarose Beads, thereby reducing background noise.

3-1. Immunoprecipitation (IP) Protocol：

1) Antibody Immobilization

①Resuspend the Protein A/G Agarose Beads suspension by pipetting up and down or inverting the tube. Aliquot 20 μL of beads into a 1.5 mL centrifuge tube per sample, add 0.5~1.0 mL of binding buffer (cell lysis buffer or PBS is also acceptable) to resuspend the beads, centrifuge at 1000 rpm for 1 minute, and aspirate the supernatant.

②Add 0.5~1.0 mL of binding buffer to the equilibrated beads, followed by 1~5 μg of normal IgG or the antibody against the target protein. Incubate with rotation at 4℃ for 1~2 hours to allow antibody immobilization. Centrifuge at 1000 rpm for 1 minute, collect the beads for subsequent use, and leave a small volume of buffer to prevent desiccation of the beads.

2) Antibody Cross-linking (Optional; skip this step if co-elution of the antibody-target antigen complex is required)

①Add 1 mL of cross-linking buffer to the washed beads, centrifuge at 1000 rpm for 1 minute, and aspirate the supernatant.

②Add 1 mL of cross-linking buffer containing 20 mM DMP (prepare fresh before use), resuspend the beads, and mix by gentle inversion or rotation at room temperature for 30 minutes to ensure full contact between the buffer and beads. Centrifuge at 1000 rpm for 1 minute, and aspirate the supernatant.

③ Add 1 mL of stop solution to resuspend the beads to terminate the cross-linking reaction, mix by gentle inversion or rotation at room temperature for 15 minutes, centrifuge at 1000 rpm for 1 minute, and aspirate the supernatant.

④Add 0.5 mL of wash buffer to resuspend the beads for washing, centrifuge at 1000 rpm for 1 minute, and aspirate the

supernatant. Repeat the washing step twice, and leave a small volume of buffer to prevent desiccation of the beads.

3) Immunoprecipitation Reaction

①**Antigen binding**: Add the antigen-containing sample (cell or tissue lysate with the target protein) to the antibody-immobilized beads, resuspend the beads thoroughly in the lysate by gentle pipetting or inversion, and incubate with gentle rotation at 4℃ for 1~2 hours to allow full antigen-antibody binding. For weak binding interactions, incubation can be performed overnight.

②**Washing**: Centrifuge the antigen-bound beads at 1000 rpm for 1 minute, collect the supernatant and place on ice for detection (or discard directly). Add 1 mL of wash buffer (cell lysis buffer is also acceptable) to the centrifuge tube, mix with the beads, and incubate with rotation at 4℃ for 5~10 minutes. Centrifuge at 1000 rpm for 1 minute, discard the supernatant, and repeat the washing step twice. Aspirate the buffer as completely as possible in the final wash.

3-2. Co-Immunoprecipitation (CoIP) Protocol：

1) Antigen-Antibody Binding：

Mix IgG or the specific antibody with the cell or tissue lysate containing the target protein, and incubate at 4℃ for at least 2 hours, or overnight.

Note：The incubation time depends on the antigen-antibody binding efficiency and the stability of the target protein, and needs to be optimized for specific experiments. The amount of antibody added should be based on the amount of beads used in subsequent steps; 1~5 μg of IgG or anti-target protein antibody is generally recommended.

2) Bead Preparation：

Aliquot 20 μL of Protein A/G Agarose Beads into a 1.5 mL centrifuge tube, add 0.5~1.0 mL of binding buffer (cell lysis buffer or PBS is also acceptable) to resuspend the beads, centrifuge at 1000 rpm for 1 minute, and aspirate the supernatant.

3) Capture of Antigen-Antibody Complexes：

Add the lysate containing the antigen-antibody complex to the equilibrated beads, and incubate with gentle rotation at 4℃ for 1~2 hours to allow full capture of the complex by the beads. Centrifuge at 1000 rpm for 1 minute, collect the supernatant for detection (or discard directly).

4) Washing：

Add 1 mL of wash buffer (cell lysis buffer is also acceptable) to the centrifuge tube, mix with the beads, and incubate with rotation at 4℃ for 5~10 minutes. Centrifuge at 1000 rpm for 1 minute, discard the supernatant, and repeat the washing step twice. Aspirate the buffer as completely as possible in the final wash.

4. Sample Elution：

1) Denaturing Elution：

This method is suitable for SDS-PAGE analysis. Add 20~40 μL of 1×SDS-PAGE Loading Buffer to the centrifuge tube with the beads, mix well, and heat at 95℃ for 5 minutes. Centrifuge at 1000 rpm for 1 minute, and collect the supernatant for subsequent Western Blot (WB) analysis.

2) Non-denaturing Elution：

Samples eluted by this method retain their native biological activity and can be used for subsequent functional assays.

Procedure: Add 5 volumes of elution buffer to the bead-antibody-antigen complex obtained from the immunoprecipitation reaction, mix gently by pipetting up and down several times, and incubate at room temperature for 10 minutes with gentle flicking or rotation. Centrifuge at 1000 rpm for 1 minute, aspirate the supernatant, and collect the eluate which contains the target protein complex. Transfer the eluate to a new centrifuge tube, and immediately add 1/10 volume of neutralization buffer to adjust the pH to 7.0~8.0 for subsequent use.