BioLinkedIn
LAB ON THE MAGNETICBEADS

Protein A Agarose

Product Description

The Biolinkedin® Protein A Agarose are typically used for isolating antibodies from serum, cell culture supernatant or ascites and for immunoprecipitation and co-immunoprecipitation of antigens from cell or tissue extracts. Protein A Agarose contain a recombinant Protein A that combines the IgG binding domains of both Protein A. Protein A contains five Fc-binding domains from Protein A. These domains can bind to antibodies from many different species, including mouse, human, rabbit, pig, dog and cat.

Product Features

Composition	Recombinant Protein A
Medium	4% Highly crosslinked agarose micr ospheres
Particle size	30-100μm
Concentration	25%
Binding Capacity	≥ 6 mg human IgG/mL of bead
Application	IP, CoIP, ChIP
Storage Condition	Store at 4°C for 2 years.

Protocol

1. Incubate cultured cells (80-90% confluent monolayer in 100 mm cell culture plate or approximately 2-5 x 10^7 suspension cells in flask).
2. Add 3 ml ice cold Biolinkedin® IP Lysis/Wash Buffer to cell monolayer and incubate at 4° C for10 minutes. For suspension cells, add the Biolinkedin® IP Lysis/Wash Buffer to washed cell pellet in a 15 ml conical centrifuge tube.
3. Disrupt cells by repeated aspiration through a 21 gauge needle and transfer to a 15 ml conical centrifuge tube.
4. Wash cell culture plate with additional 1.0 ml ice cold Biolinkedin® IP Lysis/Wash Buffer and combine with original extract.
5. Pellet cellular debris by centrifugation at 10,000 x g for 10 minutes at 4° C. Transfer supernatant to a fresh 15 ml conical centrifuge tube on ice. Preclear lysate (optional step) by adding 1.0 µg of the appropriate control IgG (normal mouse, rat, rabbit or goat IgG, corresponding to the host species of the primary antibody), together with 20-50 µl of resuspended volume of Protein A Agarose. Incubate at 4° C for 0.5h
6. Pellet beads by centrifugation at 2,500 rpm (approximately 1,000 x g) for5 minutes at 4° C. Transfer supernatant (cell lysate) to a fresh 15 ml conical centrifuge tube on ice.
7. Transfer 1 ml of the above cell lysate, or approximately 100-500 µg total cellular protein, to a 1.5 ml microcentrifuge tube. Add 1-10 µl (i.e., 0.2-2 µg) primary antibody (optimal antibody concentration should be determined by titration) and incubate for 1 hour at 4° C.
8. Add 20 µl of resuspended volume of Protein A Agarose. Cap tubes and incubate at 4° C on a rocker platform or rotating device for 1 hour to overnight.
9. Collect immunoprecipitates by centrifugation at 2,500 rpm (approximately 1,000 x g) for 5 minutes at 4° C. Carefully aspirate and discard supernatant.
10. Wash pellet 4 times with 1 ml Biolinkedin® IP Lysis/Wash Buffer (more stringent) or PBS (less stringent), each time repeating centrifugation step above.
11. After final wash, aspirate and discard supernatant and resuspend pelletin 40 µl of 1x electrophoresis sample buffer.

Troubleshooting

Q1: How to improve the efficiency of antibody binding to Agarose?

A1: The binding efficiency of Agarose to antibodies is related to the species and subtype of the antibody. Please confirm the affinity of the type of antibody with the affinity of Protein A ligand. If the affinity of the subtype of the antibody is lower, increase the incubation time of the antibody and the Agarose(30 to 120 min) and the pH of the binding buffer(8-9), and reduce the ionic strength (25~100 mM NaCl)

Q2: How to improve the specificity of Agarose in immunoprecipitation?

A2: The antibody can be incubated with the sample to form an antibody-antigen complex, and the complex can be captured with Protein A Agarose. This method can increase the binding efficiency of the antibody to the antigen and reduce the binding time of the with the sample, thereby increasing the specificity of the precipitated product. This method is also recommended for CoIP &ChIP.

Q3: How to solve the phenomenon that the Agarose are easy to adhere to the tube wall?

A3: Recommend to use a low adsorption tube for magnetic bead operation. In addition, the addition of 0.01% to 0.1% (v/v) of nonionic detergent (such as NP-40, Tween-20 or Triton X-100) into the buffer can effectively reduce the adhesion of the Agarose to the tube.

Q4: How to solve agglomeration of the during use?

A4: If the Agarose are agglomerated during use, it is generally difficult to oscillate and break up that tends to uneven distribution. The reason is that the beads are placed in the magnetic field for too long and the beads are firmly bonded together. After treated with ultrasonic water bath for 2 minutes, the can be dispersed. However it should be noted the ultrasonic treatment time.

Appendix : Binding strength of Protein A to different species of Ig·s and their subclasses.

Species	Antibody Subtype	Protein A
Human	Total IgG	+++++
	IgG1, IgG2	+++++
	IgG3	-
	IgG4	+++++
	IgM	+
	IgD	-
	IgA	+
	IgA1, IgA2	+
	IgE	+++
	Fab	+
	ScFv	+
Mouse	Total IgG	+++++
	IgM	-
	IgG1	+
	IgG2a ,IgG2b	+++
	IgG3	+++
Rat	Total IgG	+++
	IgG1	+
	IgG2a	+
	IgG2b	+
	IgG2c	+++++
Cow	Total IgG	+++
	IgG1,IgG2	+++
Goat	Total IgG	+++
	IgG1,IgG2	+++
Sheep	Total IgG	+++++
	IgG1, IgG2	+++++
Horse	Total IgG	+++++
	IgG(ab),IgG(c)	+
	IgG(T)	+
Rabbit	Total IgG	+++++
Guinea Pig	Total IgG	+++
Hamster	Total IgG	+++
Pig	Total IgG	+++++
Donkey	Total IgG	+++
Cat	Total IgG	+++++
Dog	Total IgG	+++++
Monkey	Total IgG	+++
Chicken	Total IgG	-
Notes:	+ weak binding	+++ medium binding
	+++++ strong binding	- no binding