Protein A/G Agarose

Summary

Catalog No	103-101-002
Ligand	Recombinant Protein A/G
Bead size	~ 40 μm
Binding capacity	High binding capacity, 10 μL Protein A/G Agarose bind about 50 μg of Human IgG.
Storage	Shipped at ambient temperature. Do not freeze.
Storage buffer	50 % slurry in PBS containing 20 % Ethanol

Description

Protein A/G Agarose is useful tools for purification antibodies and immunoprecipitation(IP). Samples containing IgG are incubated with Protein A/G Agarose in a buffer that facilitates binding. After non-IgG and nonantigen components of the sample are washed from the resin, and the bound IgG and antigen may be recovered by elution.

Background

Gene fusion of the Fc-binding domains of Protein A and Protein G has resulted in production of a structural and functional chimeric protein with broader binding than either Protein A or Protein G alone. During fusion, the Protein G gene sequence coding for the serum albumin-binding site is eliminated. The product obtained is consistent in quality and yield because the bacterial host is engineered to be deficient in major proteolytic activities. Binding is less pH-dependent than either Protein A or Protein G alone, occurring well at pH 5-8. The extended Fc-binding properties of Protein A/G make it a popular tool in the investigation and purification of immunoglobulins. Protein A/G binds to all human IgG subclasses, IgA, IgE, IgM and to a lesser extent IgD; however, it does not bind mouse IgA, IgM or murine serum albumin.18 Protein A/G is an excellent tool for purification and detection of mouse monoclonal antibodies from IgG subclasses without interference from these other serum proteins. Individual subclasses of mouse monoclonals are most likely to have stronger affinity to this chimeric protein than to either Protein A or Protein G.

Application notes

Immunoprecipitation (IP)/Co-IP
Protein Purification
Mass spectrometry (MS)
Enzyme activity measurements

Immunoprecipitation protocol

Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners.

For one immunoprecipitation reaction, we recommend using ~106- 107 cells.

1. Choice of lysis buffer:

* For cytoplasmic proteins, resuspend the cell pellet in 200 μL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).
* For nuclear/chromatin proteins, resuspend cell pellet in 200 μL ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), MgCl2 (f.c. 2.5 mM), protease inhibitor cocktail and PMSF(f.c. 1 mM)(not included)

2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.

3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre cooled tube and add 300 μL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 μL of diluted lysate for further analysis (input fraction).

Primary antibody binding

1.take supernant of the cell lysis into another tube and then add primary antibody (2ug-4ug/sample).

2.rotate at +4°C overnight.

Bead equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold Dilution buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
5. Discard the supernatant.

Protein binding

1. Transfer 25 µL of equilibrated beads to the tube of protein binding.
2. Rotate end-over-end for 1 hour at +4°C.

Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
2. If required, save 50 µL of supernatant for further analysis(-flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 µL Wash buffer.
5. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the remaining supernatant.
6. Repeat this step at least twice.
7. During the last washing step, transfer the beads to a new tube.

Optional: To increase stringency of the Wash buffer, test various salt concentrations e.g. 150 mM - 500 mM,and/or add a non-ionic detergent e.g. Triton™ X-100.

Elution with 2x SDS-sample buffer

1. Remove the remaining supernatant.
2. Resuspend beads in 80 µL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE.

Elution with Glycine-elution buffer

1.Remove the remaining supernatant.
2. Add 50–100 µL Glycine-elution buffer and constantly pipette up and down for 30 - 60 sec at +4°C.and down for 30 - 60 sec at +4°C.
3. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency .

Suggested buffer compositions

Buffer	Composition
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % NP40
RIPA buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1 % SDS, 1 % Triton™ X-100, 1 % deoxycholate
Dilution/Wash buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol
Glycine-elution buffer	200 mM glycine pH 2.0
Neutralization buffer	1 M Tris pH 10.4

Related products

Code Number	Product Description	Size	prices(¥)
019-101-002	GFP Nanoselector Agarose	0.25mL	1500
019-101-003	GFP Nanoselector Magnetic beads	0.25mL	1500
020-101-002	RFP Nanoselector Agarose	0.25mL	1500
020-101-003	RFP Nanoselector Magnetic beads	0.25mL	1500
013-101-002	mNeongreen Nanoselector Agarose	0.25mL	1500
014-101-002	TurboGFP Nanoselector Agarose	0.25mL	1500
015-101-002	MBP Nanoselector Agarose	0.25mL	1500
010-101-002	GST Nanoselector Agarose	0.25mL	1500
011-101-002	SNAP tag Nanoselector Agarose	0.25mL	1500
012-101-002	Halo Nanoselector Agarose	0.25mL	1500
003-101-002	HA tag Nanoselector Agarose	0.25mL	1500
004-101-002	c-His tag Nanoselector Agarose	0.25mL	1500
049-101-002	mWasabi Nanoselector Agarose	0.25mL	1500
017-101-002	TagFP Nanoselector Agarose	0.25mL	1500
025-101-002	Rabbit IgG Nanoselector Agarose	0.25mL	1500
001-101-002	Mouse IgG Nanoselector Agarose	0.25mL	1500
067-101-003	Streptavidin Magnetic beads	0.25mL	1500
100-100-100	Binding Control Nanoselector Agarose	1mL	800
100-100-200	Binding Control Magnetic beads	1mL	800