Protein A & G Spin Plates

96-well filter plates for high throughput purification and screening of antibodies

(Cat. # 786-996, 786-997)

INTRODUCTION

The Protein A and G spin plates for IgG screening are versatile, spin-format, 96-well filter plates for high throughput purification and screening of antibodies and for immunoprecipitations (IP). Each well can process 10-100µl serum, cell culture supernatant or ascites fluid.

Protein A and Protein G are for binding the constant domains of immunoglobulin (Ig) molecules, with different binding characteristics (Table 1). Protein A or G are coupled to agarose beads by reductive amination method that provides high coupling efficiency for immunoglobulins and minimal protein leaching.

Species	Antibody Class	Protein A	Protein G
Mouse	Total IgG	++++	++++
	IgG_1	+	+++
	IgG_{2a}	++++	++++
	IgG_{2b}	++++	++++
	IgG_3	+++	+++
Human	Total IgG	++++	++++
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	+	++++
	IgG_4	++++	++++
Rat	Total IgG	+	++
	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_{2c}	++	+++
Hamster	Total IgG	++	++
Guinea Pig	Total IgG	++++	++
Rabbit	Total IgG	++++	+++
Horse	Total IgG	++	++++
Cow	Total IgG	++	++++
Pig	Total IgG	+++	++
Sheep	Total IgG	+	++
Goat	Total IgG	+	++
Chicken	Total IgG	-	-

Table 1: Relative affinity of Protein A and Protein G for Immunoglobulins

ITEMS SUPPLIED

Cat. #	Description	Size	Wash/Collection Plates
786-996	Protein A Resin Spin Plate	1 plate	3 plates
786-997	Protein G Resin Spin Plate	1 plate	3 plates

STORAGE CONDITIONS

The plates are shipped at ambient temperature. Upon arrival, store at 4˚C. If stored and handled correctly the plates have a shelf-life of 1 year.

SPECIFICATIONS

Protein A

- High binding capacity: >40mg human IgG/ml resin
- Ligand: Recombinant Staphylococcal Protein A lacking the albumin-binding domain produced in E. coli
- Bead size: 45-165µm
- Bead Structure: 6% highly cross-linked agarose

Protein G

- High binding capacity: 38mg human IgG/ml resin; >20mg sheep IgG/ml resin
- Ligand: Recombinant Streptococcal Protein G lacking the albumin-binding domain produced in E. coli
- Bead size: 50-165µm
- Bead Structure: 4% highly cross-linked agarose

IMPORTANT INFORMATION

- Plates are compatible with variable speed centrifuges with rotors and carriers capable of handling stacked plates. Use speed of 500-1,000xg with a maximum of 1,000xg.
- Ensure the spin plates are balanced throughout all centrifugations with a duplicate plate filled with an appropriate volume of water.
- Each well contains 50µl of resin, which can purify 10-100µl serum, cell culture supernatant or ascites fluid.

ADDITIONAL ITEMS NEEDED

- Variable speed centrifuge with rotor and carriers capable of handling stacked plates (4.5cm height) at 500xg or a vacuum manifold.
- Multi-channel pipettor and tips
- Binding Buffer: 0.1M sodium phosphate, 0.15M sodium chloride buffer (pH 7.2)
- Neutralization buffer (1M Tris ,pH7.5-9)
- Elution Buffer (0.1M glycine, pH2)

PROTOCOL

IgG purification

1. Bring the spin plate and buffers required for binding and elution of antibodies to room temperature.
2. Remove the bottom and top seals of the spin plate. Place the bottom of spin plate on top of the wash plate. Add 200µl of Binding Buffer (0.1 M sodium phosphate, 0.15M sodium chloride buffer (pH 7.2)) to each well for equilibration.
   NOTE: *0.1 M sodium phosphate, 0.15 M sodium chloride (pH: 7.2) is appropriate buffer for equilibration, binding of antibodies to protein A/G agarose beads and for washing to remove unbound antibodies and other serum proteins.*
3. Centrifuge the spin plate for 1 minute at 1,000 x g and discard the flow through. Add 200µl of Binding Buffer and repeat the centrifugation.
4. Dilute the sample 1:1 with Binding Buffer and load the sample into the well (10-100µl).
5. Place the plate assembly on shaker at moderate speed and incubate for 1 hr at room temperature.
6. Centrifuge the plate assembly at 1,000 x g for 1 minute. Discard or collect the flow through depending on your need.
   NOTE: *If the antibody concentration is too high then save the flow through to repeat the above process to recover maximum IgGs*
   NOTE: *At this stage the wash/collection plate can be changed or reused after washing with DI water.*
7. Wash the resin by adding 400µl Binding Buffer and centrifuge at 1,000 X g for 1 min. Discard the flow through and repeat this step 2-3 times.
8. Add 10µl of Neutralization buffer (1M Tris ,pH7.5-9) to each well of a fresh collection plate.
9. Place the spin plate on the top of collection plate with Neutralization Buffer and add 100-200µl Elution Buffer (0.1M glycine, pH2) and incubate on shaker for 1 min at room temperature. Centrifuge at 1,000 x g for 1min and collect the eluted IgGs. Repeat this step once. Make small aliquots of eluted IgGs and store at -20°C for future use.
10. The spin plate can be discarded or regenerated by washing three times with 400µl of Elution buffer followed by washing three times with 400µl of 0.02% sodium azide in ultrapure water.
11. Seal the bottom of plate. Add 100µl of 0.2% sodium azide in ultrapure water add then seal the top of the plate. Place the plate in the original sealable bag and store at 4°C

Immunoprecipitation (IP)

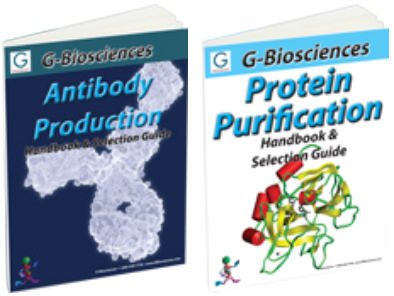
1. Make or dilute the sample containing desired antigen in 0.1 M phosphate buffer, 0.15M sodium chloride; pH:7.2. Mix appropriate amount of sample solution with antibody solution. Incubate the mixture overnight at 4°C for antigen-antibody interaction.
2. Repeat the steps described above (IgG purification, 1-11). In this case the sample would be antigen-antibody mixture and no dilution with phosphate buffer is required. The antigen-antibody complex mixture can be directly loaded on equilibrated spin plate.

TROUBLESHOOTING

Issue	Suggested Reason	Possible Solution
Antibody purification was successful but no desired antibody detected	Desired antibody is present in low concentration in the sample	Other method like affinity purify the antibody using antigen coupled to activated support can be used
Antibody of interest purified but is degraded as assessed by downstream assays	Antibody is sensitive to low pH of Elution buffer	Milder Elution buffer can be tried
	Downstream application is sensitive to Elution buffer	Desalt the eluted sample into appropriate buffer
No antibody detected in any elution fraction	Sample does not have the antibody type that binds to Protein A or Protein G	Refer to table 1 to check for antibody type detected by protein A and protein G resin

RELATED PRODUCTS

Download our Antibody Production and Protein Purification Handbooks



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