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Protein A Agarose

1 Contents

Components	HY-K0213-5 mL	HY-K0213-10 mL	HY-K0213-50 mL
Protein A Agarose (Settled Resin)	5 mL	10 mL	50 mL

2 General Information

MCE Protein A Agarose is an affinity chromatography medium for separation and purification of immunoglobulins. Protein A is a bacterial cell wall protein isolated from *Staphylococcus aureus* and binds to most mammalian IgGs mainly through Fc regions. Native Protein A contains 5 IgG binding domains and many other domains with unknown functions. The latter have been eliminated from recombinant Protein A to reduce nonspecific binding. MCE Protein A Agarose, a 4% highly cross-linked agarose reagent coupled with recombinant Protein A, effectively purifies mammalian monoclonal and polyclonal antibodies, such as human IgG, IgE, IgM.

3 Characteristics

Matrix Spherical	4% cross-linked agarose
Degree of Substitution	2 mg Recombinant Protein A/mL Settled Resin
Bead Diameter	45-165 μm
Binding Capacity	> 20 mg human IgG/mL Settled Resin
Storage Solution	50% slurry in 1× PBS containing 20% ethanol

4 General Protocol

1. Buffer Preparation

Binding/Wash Buffer	20 mM Na_2HPO_4, 0.15 M NaCl, pH 7.0
Elution Buffer	0.1 M Glycine, pH 3.0
Neutralization Buffer	1 M Tris-HCl, pH 8.5

Buffer filtration with 0.45 μm filter is recommended.

2. Sample Preparation

It is necessary to insure that the proper ionic strength and pH are maintained for optimal binding. For serum samples, ascites fluid or tissue culture supernatant, it is necessary to dilute them at least 1:1 with Binding/Wash Buffer. Alternatively, the sample may be dialyzed overnight against Binding/Wash Buffer.

3. Load of Protein A Agarose

(1) Mix the slurry by gently inverting the bottle several times to completely suspend the agarose.

(2) Transfer an appropriate-sized slurry to the column and allow the agarose to settle down and the storage buffer to drain from the column.

4. Sample Purification

(1) Equilibrate the column with 5× bed volumes of Binding/Wash Buffer with a flow speed of about 1 mL/min.

(2) Apply appropriate-sized sample to the column with a flow speed of about 0.5-1 mL/min. Collect and save the flow-through for analysis.

Note: Binding capacity is flow rate- and protein-dependent. Higher flow rates will decrease production time, but may result in losing a small portion of the target antibody.

(3) Wash the column with approximately 15-30× bed volumes of Binding/Wash Buffer with a flow speed of about 2 mL/min or until the absorbance at 280 nm is stable. If desired, save supernatant for downstream analysis.

(4) Elute with approximately 10-15× bed volumes of Elution Buffer with a flow speed of about 1 mL/min, Collect the eluate and immediately neutralize to pH 7.4 with Neutralization Buffer (1/10 volume of total eluate).

(5) Analyze the target protein by SDS-PAGE, along with fractions collected from different steps if necessary.

5. Regeneration of Column

Regenerate the column by washing the resin with 10× bed volumes of Elution Buffer or Guanidine Hydrochloride (6 M, pH 8.0) followed by equilibration with 5× bed volumes of Binding/Wash Buffer. Columns can be regenerated up to 10 times without significant loss of binding capacity.

6. Storage of Column

Store regenerated Protein A Agrose in Binding/Wash Buffer containing 20% ethanol at 2°C to 8°C. Do not freeze.

5 Storage

Store at 2-8°C, and is stable for at least 2 years.

Do not dry or freeze.

6 Precautions

1. Do not dry or freeze the agarose.
2. Binding capacity is flow rate- and protein-dependent. Higher flow rates will decrease production time, but may result in losing a small portion of the target antibody.
3. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
4. For your safety and health, please wear a lab coat and disposable gloves to operate.

7 Troubleshooting

Problem	Possible Cause	Solution
Highly backpressure	Sample was highly particulate.	Centrifuge or filter (0.45 μm) before use.
	Gas presents in buffers or sample on the column, which results in blockage of gel pores with microscopic air bubbles.	Degas buffers and remove air bubbles from column.
Considerable antibody purified, but no specific antibody of interest detected	Antibody of interest is at low concentration or has low binding affinity for the immobilized protein relative to other immunoglobulins in the sample.	Use serum-free medium for cell supernatant samples.
		Affinity-purify the antibody using the specific antigen coupled to a support.
Antibody of interest purified, but it is denatured	The antibody is sensitive to low-pH elution buffer.	Neutralize the eluted fractions with Neutralization Buffer immediately after elution.
No antibody is detected in any elution fraction.	The IgG subclass does not bind to Protein A.	Try other affinity chromatography media to purify the antibody, such as HY-K0214 Protein G Agarose or HY-K0215 Protein L Agarose.

Appendix: Binding Affinity of Protein A, Protein G, Protein L for Different Antibodies and Isotypes

Species	Antibody Subtypes	Protein A	Protein G	Protein L
Human	IgG	+++	+++	+++*
	IgG1	++++	++++	+++*
	IgG2	++++	++++	+++*
	IgG3	-	+++	+++*
	IgG4	++++	++++	+++*
	IgA	+	-	+++*
	IgA1	+	-	+++*
	IgA2	+	-	+++*
	IgD	+	-	+++*
	IgE	++	-	+++*
	IgM	+	-	+++*
	Fab	+	+	+++*
	ScFv	+	-	+++*
Mouse	IgG	+++	+++	+++*
	IgG1	+	++++	+++*
	IgG2a	+++	+++	+++*
	IgG2b	+++	+++	+++*
	IgG3	++	+++	+++*
	IgM	-	-	+++*
Rat	IgG	+	++	+++*
	IgG1	+	+	+++*
	IgG2a	+	++++	+++*
	IgG2b	+	++	+++*
	IgG2c	++	++	+++*
	IgG3	+	++	+++*
Cow	IgG	+	+++	-
	IgG1	+	+++	-
	IgG2	++	+++	-
Goat	IgG	++	+++	-
	IgG1	+	+++	-
	IgG2	+++	+++	-
Sheep	IgG	+	++	-
	IgG1	+	++	-
	IgG2	+++	+++	-
Horse	IgG	++	++++	?
Rabbit	IgG	+++	+++	+*
Guinea Pig	IgG	+++	+	?
	IgG1	++	+	?
	IgG2	++	+	?
Hamster	IgG	+	++	+*
Pig	IgG	+++	++	+++*
Donkey	IgG	++	+++	?
Cat	IgG	+++	+	?
Dog	IgG	++	+	?
Monkey	IgG	++++	++++	?
Chicken	IgG	-	-	-
Koala	IgG	-	+	?
Llama	IgG	-	+	?

Notes

+ weak binding
+++ medium binding
++++ strong binding
- no binding
* the binding strengths for Protein L refer only to antibody species and subtypes with appropriate kappa light chains. Lambda light chains and some kappa light chains do not bind.