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User's Guide

Protein A/G *Xpure* Agarose Resin

DESCRIPTION

Recombinant protein A and protein G is immobilized on 4% highly cross-linked agarose resins by means of covalent binding that avoids protein loss and allows for re-use. This product is suitable for batch or FPLC purifications of classes, subclasses and fragments of immunoglobulins from cell culture media or biological fluids.

Protein A/G *Xpure* Agarose Resin Specifications:

Ligand Density: ~ 3 mg Protein A/G /ml resin
Binding Capacity: ~ 25 mg human IgG / ml resin

Resin: 4% highly crosslinked agarose beads

Storage Buffer: 50% slurry in 1X PBS with 20% ethanol

pH Range: 3 -10

Maxi Pressure: 0.3 MPa or 3 bar

Storage Temperature: 2-8 ^{0}C

Table 1. Relative binding strength of Protein A and Protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	—
	IgD	—	—
	IgE	—	—
	IgG1	++++	++++
	IgG2	++++	++++
	IgG3	—	++++
	IgG4	++++	++++
	IgM	variable	—
Avian egg yolk	IgY	—	—
Cow		++	++++
Dog		++	+
Goat		—	++
Guinea pig	IgG1	++++	++
	IgG2	++++	++
Hamster		+	++
Horse		++	++++
Koala		—	+
Liama		—	+
Monkey (rhesus)		++++	++++

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Mouse	IgG1	+	++++
	IgG2a	++++	++++
	IgG2b	+++	+++
	IgG3	++	+++
	IgM	variable	—
Pig		+++	+++
Rabbit	no distinction	++++	+++
Rat	IgG1	—	+
	IgG2a	—	++++
	IgG2b	—	++
	IgG3	+	++
Sheep		+/-	++

++++ = strong binding; ++ = medium binding; — = weak binding or no binding

INSTRUCTIONS

A. Batch Purification

1. Eliminating the Preservative and Equilibrating Protein A/G Agarose Resins

Transfer appropriate amounts of resins to an eppendorf tube or conical tube, wash the beads with 10 resin volumes of binding buffer three times to eliminate the preservative. Pellet resins by centrifugation at 1,000 X *g* for 2 min each time.

Commonly used binding buffer: Sodium phosphate (25 mM), Tris (50 mM), or 1X PBS, pH 7.0-7.4, 150 mM NaCl, and 10% glycerol.

2. Applying the Sample

Once the resin is equilibrated, the sample containing the immunoglobulin for purification can be added into resins. Gently rock the mixture at 4 ^{0}C for 3 – 16 hours.

Note: In some cases a slight increase of contact time may facilitate binding.
Note: Sometimes diluting simple 1:1 with binding buffer before application is advisable to maintain the proper ionic strength and pH for optimal binding.
Note: Binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.

4. Washing Protein A/G Agarose Resin

After binding, wash resins with binding buffer for three times. Pellet the resin by centrifugation at 1,000 X *g* for two minutes each time. To remove non-specific binding, it is recommended to increase salt concentration to 0.5-1 M NaCl in binding buffer. Detergents such as 1% TX-100 or 0.5% NP-40 can also be added.

5. Eluting the Purified Immunoglobulin

If samples are applied for immunoblotting, appropriate amounts of 1X SDS sample buffer can be used to elute the bound IgG or co-immunoprecipitated proteins with boiling. Pellet resins by centrifugation, supernatants can be applied for SDS-PAGE.

Alternatively, elution can be achieved by reducing pH below 3.0. Common elution buffer: 100 mM glycine, pH 3.0.

Note: It is recommended to rapidly neutralize eluted samples by adding 0.15 ml 1 M Tris, pH 8.5 per ml of eluted immunoglobulin to neutralize the eluted fractions.

6. Storage *Xpure*

Protein A/G *Xpure* resins can be regenerated by washing with 10 volumes of eluting buffer (100 mM glycine, pH 3.0) three times, then rinse with distilled water. Finally restore in 1X PBS with 20% ethanol. Keep at 2-8 ^{0}C.

B. FPLC Column Purification

1. Gently shake the resin bottle several times to obtain a homogeneous suspension of **Protein A/G *Xpure* Agarose resin**/preservative. Place a funnel in the head of column and slowly run the suspension down the walls of an empty FPLC column.

   Note: It is advisable to add resins slowly to avoid the formation of air bubbles. The product may also be degassed before added to the column.

   Decant the product and discard most of the leftover liquid, leaving 1 cm above the column head to prevent drying. This is done either by passing it through the column or pipetting it from the top of the column.
2. Repeat previous steps until the desired column height is obtained.
3. Insert the adapter gently in the column head until it begins to displace the liquid.

   Note: Make sure no air is trapped under the net.
4. Add distilled water to the purification stream until a constant height (corresponding to the height of the column) is achieved.

   Note: If the desired height is not achieved, repeat steps 1 through 4.
5. When a constant height has been obtained, maintain the flow with the addition of 5 volumes of distilled water to completely eliminate the preservative.
6. Equilibrate the column with 5 to 10 column volumes of binding buffer.

   Note: It is advisable to previously de-gas all the solutions before adding to the column to avoid the formation of bubbles.
7. The packed column can be used by following the instruction of your FPLC instrument. Make sure to set the maxi pressure of the column no more than 0.3 MPa. Usually 0.5-1 mL/min flow rate can be applied.
8. After binding and washing, elute the bound IgG or proteins with elution buffer (100 mM glycine, pH 3.0). Neutralize the eluted sample with 0.15 mL 1 M Tris, pH 8.5 for each mL eluted sample. We recommend to pre-add 0.15 ml 1M Tris, pH 8.5 into each collection tube, in which 1 ml fraction will be collected.
9. Regenerate the column by washing resins with 10 column volumes of elution buffer (100 mM glycine, pH 3.0), followed by 10 column volumes of distilled water, then restore in 1X PBS with 20% ethanol.

TROUBLESHOOTING

Possible causes of problems that could appear during the purification protocol of immunoglobulins are listed below. The causes described are theoretical and it is always advisable to contact our team with your specific problem.

The table delineates the potential problems at each step in the protocol that might explain poor performance.

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
TARGET PROTEIN NOT BOUND TO THE COLUMN	Conditions in binding or elution are not the optimum ones.	- Optimize pH, flow, temperature as well as salt or ion concentration.
	Channels have formed in column bed so loaded sample runs through column without interacting with Protein A/G.	- Repack column.
	Column has not been stored in recommended conditions after previous usage.	- Always follow manufacturer recommendations.
	The antibody to be purified has low affinity with Protein A/G.	- Look up bibliography on the subject and, if that observation is true, try an alternative way of purification.

	Protease presence.	- Add protease inhibitors to sample loading / wash buffer. - Work at lower temperatures (such as 4°C) to minimize degradation.
THE ANTIBODY IS DEGRADED	Antibody can be unstable in elution conditions.	- Follow usage instructions neutralizing the fractions of the eluted antibody.
ANTIBODY IS NOT DETECTED IN THE ELUTION PROCESS	The IgG subclass doesn't bind to the resin.	- Use another affinity column to purify the antibody.
BUBBLES IN THE PRE-PACKED COLUMN	Column poured and stored at one temperature, but used at another.	- Equilibrate the column in the same temperature conditions as in usage step.
	There are air bubbles in sample or buffers.	- De-gas sample and buffers used.
COLUMN FLOW IS VERY SLOW	There are air bubbles in sample or buffers that are blocking flow through pores.	- De-gas sample and buffers used.