PROTEIN L AGAROSE BEADS

Procedure for Use

DESCRIPTION

Protein L is an immunoglobulin-binding protein that was isolated from the bacteria *Peptostreptococcus magnus* and provides a convenient way to separate immunoglobulins from a variety of sources. Now produced recombinantly. Protein L contains four immunoglobulin binding domains of the native protein.

Protein L resins may be used for the purification of IgG, IoM, IgA and IgD containing kappa light chains from various species without interfering with the antigen binding site. Besides antibody, Protein L is also suitable for binding of a wide range of antibody fragments such as Fabs, single-chain variable fragments (scFv), and domain antibodies (Dabs).

Resins are products that allow batch or column purifications of immunoglobulins containing light chains of type kappa I, III and IV in human and kappa I in mouse classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. Protein L is immobilized by means of covalent binding that avoids protein loss and allows for column re-use.

This product is supplied as a suspension of PROTEIN L Agarose Resin in 20% ethanol.

PROTEIN L Agarose Resin specifications:
Binding Capacity: ~ 10 mg human IgG / ml resin.
Resin: 4% crosslinked agarose beads

Note: The Binding capacity of Protein L Agarose Resin depends on the source of the immunoglobulin. There might be deviations in binding capacities for different immunoglobulins derived from the same species.

Binding of immunoglobulins to Protein L

SPECIES	SUBCLASS	'PROTEIN L
Human	Total IgG	+++
	IgG_1	++++
	IgG_2	++++
	IgG_3	+++
	IgG_4	++++
	IgA	+++
	IgA_1	+++
	IgA_2	+++
	IgD	+++
	IgE	+++
	IgM	+++
Cow	Total IgG	-
	IgG_1	-
	IgG_2	-
Horse	Total IgG	ND
Goat	IgG	-
	IgG_1	-
	IgG_2	-
Sheep	Total IgG	-
	IgG_1	-
	IgG_2	-
Monkey (rhesus)	IgG	ND

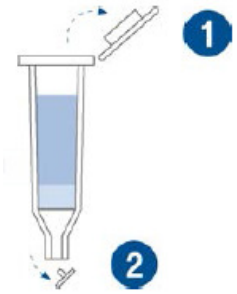
SPECIES	SUBCLASS	'PROTEIN L
Mouse	Total IgG	+++
	IgG_1	+++
	IgG_{2a}	+++
	IgG_{2b}	+++
	IgG_3	+++
	IgM	+++
Rat	Total IgG	+++
	IgG_1	+++
	IgG_{2a}	+++
	IgG_{2b}	+++
	IgG_{2c}	+++
	IgG_3	ND
Hamster		+++
Rabbit	Total IgG	+
Chicken	IgY	+
Cat	Total IgG	ND
Dog		ND
Pig		+++
Guinea-pig	IgG_1	ND
	IgG_2	ND
Koala		ND
Llama		ND

INSTRUCTIONS

I. Gravity Purification

The following summarized procedure is adapted for the purification in gravity columns.

1. Elimination of the Preservative



Determine the quantity of Protein L Agarose needed for your purification.
Gently shake the bottle of Protein L agarose to achieve a homogeneous suspension. Immediately pipette sufficient suspension to an appropriate empty column [(1)].

(1) Empty column information

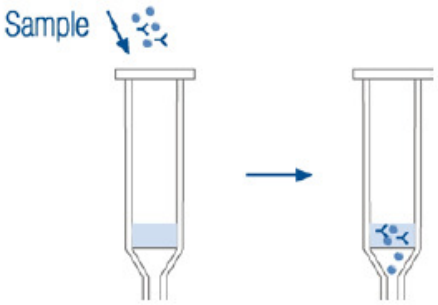
Column	Total Capacity
Plastic Columns	12ml
Plastic Columns XL	35ml

Remove first the upper and then the lower cap of the column, to allow elimination of the preservative by gravity flow.

2. Equilibration of the Resin

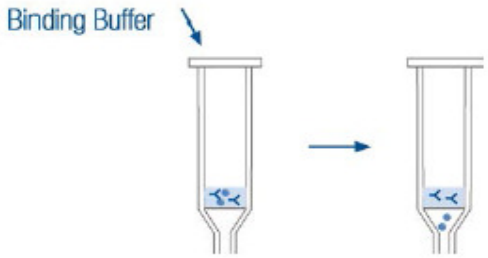
Equilibrate the column with 5ml bed volumes of binding buffer. Add the binding buffer on the upper part of the column and make sure no air has been trapped. Mix manually inverting the Pre-packed column and discard the supernatant. Repeat the equilibration step twice.
Binding buffer: 0.1M phosphate, 0.15M sodium chloride, pH 7.2.

3. Application of the Sample



Dilute sample at least with binding buffer before applying. It is advisable to maintain the Sample proper ionic strength and pH for optimal binding.
Add the sample through the top of the column keeping sample and resin in contact at least 1 hour before removing the bottom cap. Mix manually inverting the Pre-Packed column.
Remove the lower cap of the column and discard the supernatant.
Note: In some cases, a slight increase of contact time may facilitate binding.
Note: Binding capacity can be affected by several factors as sample concentration
Note: If plasma is applied, centrifuge the diluted sample at 10,000 x g for 20 minutes and add the supernatant to the equilibrated resin.

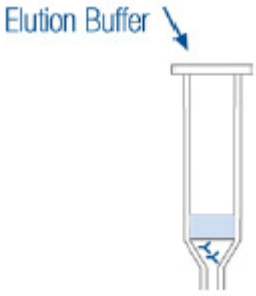
4. Washing of the Pre-Packed Column



Close column outlet with the cap. Add the binding buffer (10 bed volumes) through the top. Close column inlet with the cap, mix manually inverting the Pre-Packed column.
Remove the lower cap of the column and discard the supernatant.
Repeat the washing step twice (total wash 3 x 10 bed volumes of PBS).

Note: It will be washed with the binding buffer until the O.D. 280 nm was the same as the binding buffer.

5. Elution of Pure Immunoglobulin



Close column outlet with the cap. Add 1 bed volume of elution buffer to the column. Close column inlet with the cap and mix thoroughly for 1 0 min at room temperature. Sediment the gel, remove the end cap and collect the eluate in a new tube and store on ice.
Repeat the elution step twice and pool the collected eluates.
Elution is normally achieved at reduced pH. Most immunoglobulins are eluted in 0.1 M glycine pH 2.0 or citric acid buffer pH 2.8.
Note: It is recommended the addition of 0.15 ml of buffer pH 9.0 (e.g Tris 1M) per ml of purified immunoglobulin to neutralize the eluted fractions.
Note: Mix manually inverting the Pre-Packed column. It is advisable to keep elution buffer and resin in contact at least 10 minutes before removing the bottom cap.

6. Regeneration & Storage

Keep at +20C - +80C in a suitable bacteriostat, e.g. 20% ethanol. Do not freeze.

II. Batch Purification

The following summarized procedure is adapted for the purification in batch.

1 . Elimination of the Preservative

Determine the quantity of Protein L Agarose needed for your purification. Gently shake the bottle of Protein L Agarose to achieve a homogeneous suspension, immediately pipette the suspension (2.0 ml of the original Protein L Agarose suspension per ml of gel volume required) to an appropriate tube, Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.

2. Equilibration of the Resin

Add 10 bed volumes of binding buffer to equilibrate the gel by mixing thoroughly to achieve a homogeneous suspension. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.
Binding buffer: 0.1M phosphate, 0.15M sodium chloride; pH 7.2.

3. Application of the Sample

Once the resin is equilibrated add the sample and mix the suspension gently for 1 hour at room temperature. In some cases, a slight increase of contact time may facilitate binding. Centrifuge the suspension at 500 x g for 5 minutes to sediment the resin, carefully decant the supernatant and discard it.

4. Washing of the Resin

Wash the gel by adding 10 ml bed volumes of binding buffer. Invert to mix and centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it.
Repeat the washing step twice (total wash 3 x 10 bed volumes of binding buffer).
Note: It will be washed with the binding buffer until the O.D. 280 nm is the same as the binding buffer.

5. Elution of the Pure antibody

Add 1 bed volume of elution buffer to the gel. Mix thoroughly for 10 min at room temperature. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant or pipette the supernatant in a new tube and store on ice. Repeat the elution step twice or more and pool the fractions containing the purified protein.
Elution is normally achieved at reduced pH. Most immunoglobulins are eluted in 0.1 M glycine pH 2.0 or citric acid buffer pH 2.8.
Note: It is recommended the addition of 0.15 ml of buffer pH 9.0 (e.g Tris 1 M) per ml of purified immunoglobulin to neutralize the eluted fractions.

6. Regeneration & Storage

See the Procedure at the end of this publication.
Keep at +20C - +80C in a suitable bacteriostat, e.g. 20% ethanol. Do not freeze.

Troubleshooting Guide

Problems and Solutions

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 L.	• Re-pack column.
	• Column has not been stored in recommended conditions	• Always follow manufacturer recommendations.

	alter previous usage. • The antibody to be purified has low affinity with Protein L. • Protease presence.	• Look up bibliography on the subject and, if that observation is true, try an alternative way of purification. • Add protease inhibitors to sample loading / wash buffer. • Work at lower temperatures (such as 40C) 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. • There are air bubbles in sample or buffers.	• Equilibrate the column in the same temperature conditions as in usage step. • 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.

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