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ProductInformation

PROTEIN L - AGAROSE
From *Peptosteptococcus magnus*,
Recombinant, Expressed in *E. coli*

Product Number **P 3351**

Product Description

Protein L - Agarose is prepared with recombinant Protein L, from *Peptostreptococcus magnus*, expressed in *E. coli*, cross-linked to 4% beaded agarose using cyanogen-bromide chemistry. Protein L is attached at 1-2 mg protein per ml of resin.

Protein L - Agarose provides a convenient way to separate immunoglobulins from a variety of sources. rProtein L - Agarose may be used for the purification of IgG, IgM, IgA, IgE, and IgD containing kappa (κ) light chains. It may be used to purify Fab and scFv fragments containing kappa (κ) light chains. It may be used to directly purify monoclonal antibodies from media supplemented with BSA or FCS. Also, it may be used to directly purify human or mouse antibodies from goat, bovine or sheep proteins.

Protein L from *Peptostreptococcus magnus* binds immunoglobulins (Ig) primarily through kappa (κ) light chain interactions without interfering with the antigen binding site of Igs.[1] This means that Protein L binds to a wider range of Ig classes and subclasses from a variety of species than any other commercially available Ig binding protein. Protein L does not bind to bovine, sheep or goat Ig's. A comprehensive list of binding properties is found in the table below. rProtein L is a recombinant form of the native protein and contains four Ig binding domains (B-domains).[2]

Reagents

rProtein L - Agarose is supplied as a 50% slurry in 10 mM phosphate buffer, pH 7.2, containing 0.12 M sodium chloride and 22% ethanol.
Bead size is 45-165 μm.

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Do Not Freeze. Protein L - Agarose is stable in the pH range of 2-11.

Procedure

PBS: 10 mM phosphate buffered saline, pH 7.4 (Product No. P 3813).
Elution Buffer: 0.1 M glycine, pH 2.0, or 0.2 M citrate buffer, pH 2.8.

1. Wash 1 ml of settled Protein-Agarose (P 3351) with at least 5 volumes of PBS.
2. Dilute 1 ml plasma with 9 ml PBS.
3. Mix diluted plasma with Protein L- Agarose and incubate with gentle end over end mixing for 1 hour at room temperature.
4. Pack the slurry in a column and drain.
5. Wash away unbound proteins with 10-15 column volumes of PBS.
6. Elute bound protein with 5 ml elution buffer.
7. Neutralize eluted material with Tris-base to achieve pH 7.5.
8. Analyze by SDS-PAGE.
9. Regenerate resin by washing with 5 ml elution buffer. Store resin in PBS with 15 mM sodium azide. Resin may be re-used several times.

Results

Binding capacity is approximately 3-10 mg human IgG per ml of gel.

Binding affinity is 2-3 x 10^9 M^{-1} to human IgG (free protein).[3]

Product Profile

Binding of Immunoglobulins to Protein L, Protein A and Protein G.

Species	Immuno-globulin	Binding Affinity		
		Protein A[4-7]	Protein G[8-11]	Protein L[12-13]
Human	IgG (Normal)	++++	++++	++++
	IgG1	++++	++++	++++
	IgG2	++++	++++	++++
	IgG3	–	++++	++++
	IgG4	++++	++++	++++
	IgM	–	–	++++
	IgA	–	–	++++
	IgE	–	–	++++
	IgD	–	–	++++
	Fab	++	++	++++
	Kappa	–	–	++++
	lambda	–	–	–
	ScFv	++	–	++++
Mouse	IgG1	+	++++	++++
	IgG2a	++++	++++	++++
	IgG2b	+++	+++	++++
	IgG3	++	+++	++++
Rat	IgG1	–	+	++++
	IgG2a	–	++++	++++
	IgG2b	–	++	++++
	IgG2c	+	++	++++
Bovine	IgG	++	++++	–
Cat	IgG	++++	–	NA
Chicken	IgG	–	+	++++
Dog	IgG	++++	++++	NA
Goat	IgG	+/–	++	–
Guinea Pig	IgG	++++	++	++
Hamster	IgG	+	++	++++
Horse	IgG	++	++++	+/–
Pig	IgG	+++	+++	++++
Rabbit	IgG	++++	+++	+
Sheep	IgG	+/–	++	–

Binding of Protein L to Various Immunoglobulin Light Chains.

Species	Protein L Binding
Human kappa I	++++
Human kappa II	–
Human kappa III	++++
Human kappa IV	++++
Human lambda I-IV	–
Human lambda IV	+
Mouse kappa I	++++
Mouse kappa II	–
Mouse kappa V	+

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13. It should be noted that protein L is restricted to specific subclasses of the VL domain. Thus, the affinity indicated in the table is not general for the IgG subclass, but accounts only for those antibodies carrying the right kappa light chain.

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