

Product Information

PROTEIN LA – PEROXIDASE LABELED

Product Number **P 1357**

Product Description

Protein LA, a novel hybrid protein, is constructed by fusing four of the IgG_{Fc}- and Fab-binding regions of the staphylococcal Protein A with four of the Ig κ light chain-binding domains of peptostreptococcal Protein L, is recombinantly expressed in *E. coli*¹ and conjugated to horseradish peroxidase.

Protein LA combines the binding properties of both Protein L and Protein A and in some cases gives higher affinity binding than either protein alone. The binding of an immunoglobulin to recProtein L domains does not interfere with binding of a different Ig to the Protein A domains and vice versa. Protein LA can be used to bind antibodies and antibody fragments from a wide range of species.

Protein A from *Staphylococcus aureus* has five domains that mediate interaction with the Fc region of most mammalian IgGs. Protein A also binds to the Fab region of a subset of Ig with heavy chains belonging to the V_HIII family. Protein L from *Peptostreptococcus magnus* binds immunoglobulins (Ig) primarily through kappa (κ) light chain interactions without interfering with the antigen binding site of Igs. Specifically it has been shown to bind strongly to human κ light chain subclasses I, II and IV and to many κ chains from other species such as rat and mouse. This means that Protein LA binds to the widest range of Ig classes and subclasses from a variety of species than any other commercially available Ig binding protein. A comprehensive list of binding properties is found in Table 1.

- Binds to all classes of Ig (IgG, IgM, IgA, IgE, IgD)
- Binds to the variable domain of κ light chains, especially human κ subclasses I, III and IV and mouse κ subclass I and to V_HIII heavy chains.
- Binds to the FC portion of IgG

Reagents

Protein LA-Peroxidase Conjugate is supplied as a solution in 0.01 mM phosphate buffered saline, pH 7.2,

containing 50% glycerol. Conjugate concentration is approximately 1 mg/ml.

Storage/Stability

Store at 0 to -20 °C. Stable for at least 6 months.

Procedure

ELISA

Microtiter wells are saturated with antigen.

Antigen specific scFv antibody is added.

Serial dilutions of Protein LA – Peroxidase are added to the wells.

Substrate is added, and the color conversion is read at 405 nm.

Results

By ELISA, a 1:5,000 dilution of Protein LA -Peroxidase will give an absorbance of greater than 2.

References

1. Svensson, H.G., et al., Eur. J. Biochem., **258**, 890-896 (1998).
2. Kronvall, G., et al., J. Immunol., **104**, 140 (1970).
3. Richman, D.D., et al., J. Immunol., **128**, 2300 (1982).
4. Langone, J.J., J. Immunol. Meth., **24**, 269 (1978).
5. Ey, P.L., et al., Biochemistry, **15**, 429 (1978).
6. Kronvall, G., J. Immunol., **111**, 1401 (1973).
7. Åkerström, B., and Björck, L., J. Biol. Chem., **261**, 10240 (1986).
8. Åkerström, B., J. Immunol., **135**, 2589 (1985).
9. Björck, L., and Kronvall, G., J. Immunol., **133**, 969 (1984).
10. De Chateau, M., et al., Scand. J. Immunol., **37(4)**, 399 (1993).
11. It should be noted that protein L is restricted to specific subclasses of the V_L 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.

Table 1

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

Species	Immuno- globulin	Binding Affinity			
		Protein A ²⁻⁵	Protein G ⁶⁻⁹	Protein L ¹⁰⁻¹¹	Protein LA ¹
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	NA
Chicken	IgG	-	+	++++	++
Dog	IgG	++++	++++	+	++
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	+

lpg 2/00

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.