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Well-Coated™ Protein L

96-well plates coated with immunoglobulin binding Protein L

INTRODUCTION

Well-Coated™ Protein L plates are designed to bind the kappa light chains of immunoglobulins without interfering with the antigen binding site. The Well-Coated™ Protein L plates offer greater range of immunoglobulin classes and subclasses compared to Protein A, Protein G and Protein A/G. Protein L will bind to all classes of IgG, including IgG, IgM, IgA, IgE and IgD, and binds to single chain variable fragments (scFv and Fab fragments).

Well-Coated™ Protein L plates are for single antibody assays and are not suitable for multiple assays (sandwich ELISAs) as the first antibody will not block all IgG binding sites and therefore false positives will occur with the second antibody. The wells are coated to a 100µl depth and are supplied pre-blocked in our proprietary Superior™ Blocking Buffer. The clear, white and black plates are offered for colorimetric, chemiluminescence and fluorescent detection systems, respectively.

KIT COMPONENTS

Cat. #	Components	Size
786-736	Well-Coated™ Protein L Coated 96 well plate	5 plates
786-737	Well-Coated™ Protein L Coated 8-well strip plate	5 plates
786-776	Well-Coated™ Protein L Coated 96 well plate, Black	5 plates
786-777	Well-Coated™ Protein L Coated 96 well plate, White	5 plates

STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store unopened at 4°C. Once opened the plates can be stored in a resealable bag (ZipLoc) with an appropriate desiccant at 4°C.

IMPORTANT PROTEIN L INFORMATION

- Only binds to immunoglobulins with type kappa I, III and IV in human and kappa I in mouse (see appendix)
- May be specific for certain kappa subgroups in other species
- Binds scFv without interfering with antigen binding
- Has weak binding affinity for rabbit immunoglobulins
- No binding affinity for bovine, goat or sheep immunoglobulins
- No binding affinity for lambda light chains

PROTOCOL

The following protocol is a simple ELISA protocol and the protocol and reagents used will have to be optimized for specific applications and assays.

ITEMS NEEDED BUT NOT SUPPLIED

- Antibody to be bound to plate (see Appendix for correct Well-Coated™ plate to be used).
- Wash Buffer: femtoTBST™ (Cat. # 786-161) or femtoPBST™ (Cat. # 786-162); 10X concentrated wash buffers supplemented with Tween® 20. Or an appropriate wash buffer of choice.
- Blocking Buffer for dilution: A suitable blocking buffer, we recommend our Superior™ Blocking Buffer (Cat. # 786-655 to 786-661) or NAP-BLOCKER™, an animal free blocking agent suitable for ELISA (Cat. # 786-190).



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- Labeled Antigen, visit www.GBiosciences.com for horseradish peroxidase (HRP), alkaline phosphatase (AP) and biotin labeling kits.
- Detection system for label, femtoELISA™ is a chromogenic detection system for HRP and AP (Cat. # 786-110 to 786-113)

Basic ELISA Assay

1. Wash the wells to be used three times with 200µl Wash Buffer.
2. Dilute the antibody to be bound to ~1µg/ml with the Blocking Buffer. Add 100µl to each well.
3. Incubate at room temperature for 30-60 minutes, for optimal binding use a plate shaker.
4. Wash each well with 200µl Wash Buffer.
5. Add the labeled antigen at a concentration of ~0.1µg/well, diluted in Blocking Buffer, if necessary.
6. Incubate at 37°C for 1 hour.
7. Detect the label signal according to the manufacturer's instructions

NOTE: For biotin, incubate the plate for a further 1 hour at 37°C with an enzyme-labeled streptavidin or other biotin detection system. Wash as before and then detect the signal.

APPENDIX

Species	Antibody Class	Protein L	Species	Antibody Class	Protein L
Mouse	Total IgG	*****	Human	Total IgG	*****
	IgM	*****		IgG ₁	*****
	IgG ₁	*****		IgG ₂	*****
	IgG _{2a}	*****		IgG ₃	*****
	IgG _{2b}	*****		IgG ₄	*****
	IgG ₃	*****		IgM	*****
Rat	Total IgG	*****		IgD	*****
	IgG ₁	*****		IgA	*****
	IgG _{2a}	*****		Fab	*****
	IgG _{2b}	*****		ScFv	*****
	IgG _{2c}	*****	Goat	Total IgG	-
Cat	Total IgG	?		IgG ₁	-
Chicken	Total IgY	-		IgG ₂	-
Cow	Total IgG	-	Dog	Total IgG	?
	IgG ₁	-	Guinea Pig	Total IgG	?
	IgG ₂	-	Pig	Total IgG	*****
Horse	Total IgG	?	Rabbit	Total IgG	**
	IgG(ab)	?	Sheep	Total IgG	-

Table 1: Relative affinity of Protein L for Immunoglobulins.

The strength of binding only refers to immunoglobulins with the appropriate kappa light chains.

RELATED PRODUCTS

For a wide range of ELISA products, including blocking buffers, wash buffers and other Well-Coated™ plates visit www.GBiosciences.com for more details.