Well-Coated™ Protein A, Protein G & Protein A/G

96-well plates coated with immunoglobulin binding Protein A, Protein G or Protein A/G

INTRODUCTION

Well-Coated™ Protein A, Protein G and Protein A/G plates are designed to bind the constant (F_c) region of immunoglobulins ensuring that the antigen binding domain of the antibody is orientated away from the plate, offering maximum exposure of the binding site. The immunoglobulin orientation on the *Well-Coated*™ Protein A, Protein G and Protein A/G plates improves the antibody capacity compared to plates that are coated directly with antibodies.

The choice of plate is dependent on the antibody type to be used. See the appendix a table highlighting the binding affinity for Protein A, Protein G and Protein A/G.

Well-Coated™ Protein A, Protein G and Protein A/G 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 *Superior*™ Blocking Buffer that contains a antiginically non-determining protein. The clear, white and black plates are offered for colorimetric, chemiluminescence and fluorescent detection systems, respectively.

KIT COMPONENTS

Cat. #	Components	Size
786-730	*Well-Coated*™ Protein A Coated 96 well plate	5 plates
786-731	*Well-Coated*™ Protein A Coated 8-well strip plate	5 plates
786-770	*Well-Coated*™ Protein A Coated 96 well plate, Black	5 plates
786-771	*Well-Coated*™ Protein A Coated 96 well plate, White	5 plates
786-732	*Well-Coated*™ Protein G Coated 96 well plate	5 plates
786-733	*Well-Coated*™ Protein G Coated 8-well strip plate	5 plates
786-774	*Well-Coated*™ Protein G Coated 96 well plate, Black	5 plates
786-775	*Well-Coated*™ Protein G Coated 96 well plate, White	5 plates
786-734	*Well-Coated*™ Protein A/G Coated 96 well plate	5 plates
786-735	*Well-Coated*™ Protein A/G Coated 8-well strip plate	5 plates
786-772	*Well-Coated*™ Protein A/G Coated 96 well plate, Black	5 plates
786-773	*Well-Coated*™ Protein A/G 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.

BINDING CAPACITY

***Well-Coated*™ Protein A:** ~4pmol rabbit IgG/well
***Well-Coated*™ Protein G:** ~2pmol rabbit IgG/well
***Well-Coated*™ Protein A/G:** ~5pmol rabbit IgG/well

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).
- 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 A	Protein G	Protein A/G
Mouse	Total IgG	*****	*****	*****
	IgM	-	-	-
	IgG_1	*	***	***
	IgG_{2a}	*****	*****	*****
	IgG_{2b}	*****	*****	*****
	IgG_3	*****	*****	*****
Rat	Total IgG	*	***	***
	IgG_1	*	***	***
	IgG_{2a}	-	*****	*****
	IgG_{2b}	-	*	*
	IgG_{2c}	*****	*****	*****
Cat	Total IgG	*****	*	*****
Chicken	Total IgY	-	-	-
Cow	Total IgG	*	*****	*****
	IgG1	*	*****	*****
	IgG2	*****	*****	*****
Horse	Total IgG	*	*****	*****
	IgG(ab)	*	-	*
	IgG(c)	*	-	*
	IgG(T)	-	*****	*****

Species	Antibody Class	Protein A	Protein G	Protein A/G
Human	Total IgG	*****	*****	*****
	IgG_1	*****	*****	*****
	IgG_2	*****	*****	*****
	IgG_3	*	*****	*****
	IgG_4	*****	*****	*****
	IgM	*	-	*
	IgD	-	-	-
	IgA	*	-	*
	Fab	*	*	*
	ScFv	*	-	*
Goat	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****
Dog	Total IgG	*****	*	*****
Guinea Pig	Total IgG	*****	*	*****
Pig	Total IgG	*****	*	*****
Rabbit	Total IgG	*****	*****	*****
Sheep	Total IgG	*	*****	*****
	IgG_1	*	*****	*****
	IgG_2	*****	*****	*****

Table 1: Relative affinity of Protein A, Protein G and Protein A/G for Immunoglobulins

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.

LU 02/15/2010 CMH