

FT-408992

INTERFINE CHEMICALS ANALYTICAL SCIENCES BIOCHROMATOGRAPHY BIOSCIENCES

Protein A, G, L immunoreagents

Unlabeled Protein	Protein A	Protein G	Protein L
(unlabeled)	UP275757 (purified)	75194A, 1mg	56874A, 1mg
	UP40290A (recombin.)	75194B, 5mg	(recomb.)
Labeled Protein	Protein A conj.	Protein G conj.	Protein L conj.
HRP (peroxidase)	408992, 1mg	751952, 1mg	L79611
AP (Alkaline Phosphatase)	804102, 1mg	80413A, 0.5mg	
Biotin	303970 Inquire	43791A Inquire	BI0300
FITC [492/520nm]	408974, 2mg	43853A, 1mg	
SR101 [596/620nm]	692512, 1ml		
TRITC [550/570nm]	685652, 1ml	438671, Inquire	
R-PE (PhycoErythrin) [488/580nm]	CE8010 Inquire	CE8030 Inquire	
APC (AlloPhycoCyanin) [633nm/670nm]		CE8020 Inquire	

Form: Lyophilized

Storage: : +4°C (unlabeled: -20°C possible for long term storage (M)) (do not freeze labelled reagents(H))

- Protein A, G and L are binding to IgGs from many species (see below comparison table).
- Unlabeled products are used typically for coating purpose in solid-phase immunoassays.
- Labeled Proteins G, A and L are used as secondary detection immunoreagents in a variety of immunological applications such as ELISA, IHC/IH tissue staining, immunoblotting, MicroArrays or FCM.
- The HRP, AP labels convert specific substrates in products that can be detected easily (colori- or fluori-metry)
- The Fluorescent labels (FITC, SR101, TRITC, RPE, APC) are detected upon illumination at their characteristic wavelengths [max abs./em. wavelength]

Scientific and Technical Information

IgG binding relative binding affinity and specificity to Protein A, G and L

Data compiled from different sources. Main distinctive benefit are indicated on grey :

Species		Protein A FT- <u>75194A</u>	Protein G FT- <u>75194A</u>	Protein L FT- <u>56874A</u> .
Human IgGs (total) IgG1 IgG2 IgG3 IgG4 IgA IgA1 IgA2 IgD IgE IgM scFv	IgGs (total)	+++	+++	+++
	IgG1	+++	++++	++++
	IgG2	+++	++++	++++
	IgG3	- /+	+++	+++
	+++	++++	++++	
		+	<mark>-</mark>	+++
	IgA1	+	<mark>-</mark>	+++
	IgA2	++	<mark>-</mark>	+++
	<u>IgD</u>	- /+	-	+++
	_/+	<u>-</u>	+++	
	+/++	<u>-</u>	+++	
		+	<mark>-</mark>	+++



408992				
Species		Protein A FT- <u>75194A</u>	Protein G FT- <u>75194A</u>	Protein L FT- <u>56874A</u> .
Mouse	IgG IgG1 IgG2a IgG2b <u>IgG3</u>	+++ + +++ +++	+++ ++ +++ +++	+++ +++ +++ +++
	IgM	- /+	-	+++
Rabbit	IgG	+++	+++	+
Bovine (Cow)	IgG IgG1 IgG2	+ + +++	+++ +++ +++	- - -
Cat	IgG	+++	+	?
Chicken	IgY	- /+	-	-
Dog (canine)		++	+	?
Donkey		++	+++	?
Horse	IgG	++	+++	?
Goat	IgG IgG1 IgG2	+ + +++	++ +++ +++	- - -
Guinea-pig	IgG1 IgG2	+++	+ +	?
Hamster		++	++	+++
Koala		-	+	?
Llama		-	+	?
Monkey(rhesus)	IgG	+++	+++	?
Pig (Swine)	IgG IgA (some) IgM (some)	+++ - +++	++ + +	+++ ? ++ ? ++
Rat	IgG IgG1 IgG2a IgG2b IgG2c <u>IgG3</u> IgM	+ -/+ - - ++ +	++ + ++ + ++ ++	+++ +++ +++ + +++ ? ?
Sheep	IgG IgG1 IgG2	+ + +++	++ ++ +++	- - -

Strong binding +++, medium interaction ++, weak + or no interaction -.



FT-408992

Protein A

Protein A is a highly stable surface receptor produced by *Staphylococcus aureus*, of 42 kD in its native form which is capable of binding the Fc portion of immunoglobulins, especially IgGs, from a large number of laboratory or domestic species (Boyle, 1987).

As Protein A does not bind bovine IgGs, it is taken to good account in detection and purification systems of monoclonal antibodies from FCS supplemented culture media (where Protein G co-binds unspecific bovine IgGs). However, several monoclonal antibodies do not bind to Protein A, especially the majority of rat immunoglobulins and mouse IgG1.

See more protein A information properties in sheet FT-40290A.

Protein G

Protein G is a highly stable surface receptor from *Streptococcus* sp. Lancefield Group G, which is capable of binding the Fc portion of immunoglobulins, especially IgGs, from a larger number of species than ProteinA does (in particular with Bovine, Goat and Sheep IgGs).

As Protein G does not bind to human immunoglobulin classes (IgA, IgE, IgM, IgD), nor Mouse IgM, IgA, IgE nor to serum albumin, it is useful for specific detection and high purity one-step purification from serum, and very IgG specific serological detections.

See more protein G information in sheet FT-75194A.

Protein L

Protein L (MW 40.5 kDa for recombinant protein) binds immunoglobulins (Ig) primarily through kappa (κ) light chain interactions without interfering with the antigen-binding site of Igs 1 . This recognition of certain κ -light chains means that rProtein L can bind to a wider range of Ig classes and subclasses from a variety of species than any other Ig binding protein. It is in particular useful for Human IgA/D/E/M, Mouse IgG1 and IgM, Hamster and Rat Abs.

See more protein L information in sheet FT-<u>56874A</u>.

Labels

Protein A/G/L – enzymes conjugates (**HRP**, **ALP**) are designed for immunoassays with a variety of substrates, colorigenic (i.e. OPD, TMB), fluorigenic (e.g. ADHP) or luminogenic (e.g. Luminol).

Protein A/G/L labeled by fluorescent dye (FITC, TRITC,...) are designed for fluorescent immunoassays. Protein A/G/L – **biotin** conjugates provide a convenient way to detect or purify immunoglobulins from a variety of sources utilising the specific high-affinity interaction between biotin and (strep)tavidin immunoreagents.

Guide lines for use

Reagents preparation

Protein A/G/L Lyophilized materials: after reconstitution with ultrapure water or PBS, aliquot and store at +4°C (do not freeze labelled reagents).

Coating Protein A/G/L to polystyrene microplates

Non labeled products are used typically for coating purpose in solid-phase immunoassays.

1. Dissolve Protein A/G/L (unlabeled) at 5-10µg/ml in 100 µl of 0.1M sodium bicarbonate buffer (pH 9.6).

Note: 2-3 well of a 96-well plate can incubated with buffer only for control.

- 2. Incubate over night in refrigerator (or 2 hr RT).
- 3. Wash wells with PBS and block with 200 µl of 1% BSA (v/v) in PBS or TBS for 2 Hrs.
- 4. Perform a final wash with TBS with 0.1% Tween (v/v). Plates can be used immediately or in following days.





FT-408992

Labeled Protein A/G/L

Conjugated Proteins G, A and L are used as secondary detection immunoreagents in a variety of immunological applications such as ELISA, IHC/IH tissue staining, immunoblotting, MicroArrays or FCM. The concentration of use depends on the technique, and should be calibrated for each application.

For example, the recommended dilution for the Protein G/A/L – HRP for ELISA or western blotting is a dilution to 0.5 μ g/ml with PBS buffer. It can be expected that the dilutions will vary with the different IgG subclasses, IgG species and detection label/method.

Literature - Protein A/G/L ImmunoAssays

Kincaid, R. L. and M. S. Nightingale. A rapid non-radioactive procedure for plaque hybridization using biotinylated probes prepared by random primed labeling. BioTechniques 6:42–49 (1988)

O'Shannessey, D. J., Voorstad, P. J. and R. H. Quarles. Quantitation of glycoproteins on electroblots using the biotin-streptavidin complex. Analyt. Biochem 163:204–209 (1987).

Tijssen, P. Practice and Theory of Enzyme Immunoassays. (H. Burden and P. H. van Knippenberg, eds.). Elsevier Science Publishers, Amsterdam The Netherlands (1985).

Associated documents and products

Other labels: inquire. Agarose conjugates: see 52746G.

*HRP Substrates:

TMB solution #UP664780, Op-Metal Enhanced DAB kit # 679921, UptiLight HRP chemiluminescent substrates #58372A

*AP Substrates:

pNPP tablets #UP73250, VisiGlo AP chemiluminescent substrate #BV3031

•Other Ig Binding protein: Immobilised supports Labeled proteins A, G, L

Protein A #40290AProtein A-agarose #UP49981Protein G #75194A.Protein G-agarose #UP75196Protein L #56874AProtein L-agarose #UP52746

Elution buffers (for IgG, #Q99542)

• <u>Saturating agents</u>: Carbonate coating Buffer #<u>R16490</u>

BSA agents: 30% solution #<u>UP900101</u>, powders #<u>Q84171</u>, BSA-containing <u>PBS&TBS</u> buffers

SeaBlock agent #<u>UP40301A</u> (to reduce background due mammalian crossreactivities)

See BioSciences Innovations catalogue and e-search tool.

Other information

For use in vitro research use only, not for diagnostic. Not for use in diagnostic or therapeutic procedures.

For any information, please contact your local distributor.

213 av.J.F.Kennedy, 03103 Montlucon, fax :04 70 03 82 60, hotline Interbiotech : 04 70 03 73 06

rev : S1E-M11E-H05E