Protein A and Protein G Conjugates

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Abs/Em: See Table 1

Introduction

Molecular Probes offers protein A recombinant conjugated to our Alexa Fluor® dyes (Table 1). Protein A is a bacterial protein that binds with high affinity to the Fc portion of various classes and subclasses of immunoglobulins from a number of species (Table 2). Our protein A conjugates are prepared with highly purified 45,000-dalton recombinant protein A that is free of staphylococcal enterotoxins. Like native protein A from the cell wall of *Staphylococcus aureus*, our protein A conjugates contain four potential Fc binding sites, two of which may be occupied at one time.[1-4]

Table 1. Spectral characteristics of protein A and protein G conjugates.

Cat #	Conjugate	Abs *	Em *
Protein A Conjugates			
P11047	Alexa Fluor 488	495	519
P11048	Alexa Fluor 532	531	554
P11049	Alexa Fluor 546	556	573
P11050	Alexa Fluor 568	578	603
P11051	Alexa Fluor 594	590	615
P21462	Alexa Fluor 647	650	668
Protein G Conjugates			
P11065	Alexa Fluor 488	495	519
P21041	Horseradish peroxidase	NA	NA

* Absorption (Abs) and fluorescence emission (Em) maxima, in nm. Complete spectra for these dyes are available at our website. NA = not applicable.

Table 2. Binding profiles of protein A and protein G.

Antibody	Protein A	Protein G
Cat	++	–
Chicken	–	–
Cow	+	++
Dog	++	+
Goat	+	++
Guinea pig	+	++
Horse	–	++
Human IgG_1, IgG_2, IgG_4	++	++
Human IgG_3	–	++
Human IgM, IgA, IgE	++	–
Human IgD	–	–
Mouse IgG_1	–	++
Mouse (others)	++	++
Pig	++	++
Rabbit	++	++
Rat	–	+
Sheep	–	++

++ Strong binding. + Moderate binding. – Weak or no binding.

Protein G conjugates are especially valuable for detecting human IgG_3, mouse IgG_1 and immunoglobulins from rat, sheep and horse — all of which bind poorly to protein A (Table 2). The protein G that we use to prepare our Alexa Fluor 488 conjugate and horseradish peroxidase (HRP) conjugate has been engineered so that it no longer contains either the albumin and Fab binding domains or the membrane-anchoring regions found in native protein G (from β-hemolytic *Streptococcus* of the C or G strain).[5,6] This recombinant protein G has three Fc binding sites and a molecular weight of 20,000 daltons, though its apparent molecular weight on an SDS-polyacrylamide gel is about 32,000 daltons — a characteristic of protein G and its derivatives.

Materials

Protein A and protein G conjugates are supplied as lyophilized powder in unit sizes of 1 mg. The degree of labeling is approximately 2 to 6 dye molecules per protein; the exact degree of labeling is indicated on the product's label. For the protein G–HRP conjugate, the protein G to HRP ratio is approximately 1:1. When stored desiccated at ≤–20°C, these lyophilized products are stable for at least one year. Solutions can be made by dissolving the powder in phosphate-buffered saline, pH 7.2 (PBS). Store the solutions of the fluorescent conjugates at 4°C with the addition of sodium azide to a final concentration of 2 mM; under these conditions, the solutions are stable for several months. For longer storage, divide the solutions into aliquots and freeze at ≤–20°C. PROTECT CONJUGATES FROM LIGHT. AVOID REPEATED FREEZING AND THAWING. Store solutions of the protein G–HRP conjugate at 4°C with the addition of thimer-osal to a final concentration of 0.02%. DO NOT FREEZE.

Application

It is a good practice to centrifuge the protein conjugate solution briefly in a microcentrifuge before use; only the supernatant should then be added to the experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining.

Because staining protocols vary with application, the appropriate dilution of the protein A or protein G conjugate should be determined empirically.[7]

Properties

Peak absorption and fluorescence emission wavelengths for each of the fluorescent protein A and protein G conjugates are listed in Table 1.

References

1. Adv Immunol 32, 157 (1982); **2.** Trends Biochem Sci 7, 74 (1982); **3.** J Histochem Cytochem 27, 1438 (1979); **4.** J Immunol Methods 20, 241 (1978); **5.** *Bacterial Immunoglobulin-Binding Proteins, Microbiology, Chemistry and Biology*, Vol I, M. Boyle, ed. Academic Press, 1990; **6.** J Immunol 133, 969 (1984); **7.** Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1988).

Product List *Current prices may be obtained from our website or from our Customer Service Department.*

Cat #	Product Name	Unit Size
P11047	protein A, Alexa Fluor® 488 conjugate	1 mg
P11049	protein A, Alexa Fluor® 546 conjugate	1 mg
P11050	protein A, Alexa Fluor® 568 conjugate	1 mg
P11051	protein A, Alexa Fluor® 594 conjugate	1 mg
P21462	protein A, Alexa Fluor® 647 conjugate	1 mg
P11065	protein G, Alexa Fluor® 488 conjugate	1 mg
P21041	protein G, horseradish peroxidase conjugate	1 mg

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

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