



## Protein A-Horseradish peroxidase conjugate

850-0005 Protein A-HRP 0.5ml

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### Introduction

Protein A has a high affinity for the Fc regions of IgG molecules and may be used in enzyme-conjugate form to detect primary antibodies in immunochemical procedures such as ELISA, western blotting, and immunocytochemistry. Protein A binds to immunoglobulins from a variety of species (see Appendix 1) and is an extremely versatile detection reagent.

Protein A-HRP is prepared by conjugating purified protein A to highly active HRP enzyme. The conjugate has been optimized for enhanced performance using Innova's Lightning-Link conjugation technology<sup>1</sup>.

### QC specification

Detection of rabbit IgG by ELISA: a 1/10,000 dilution of conjugate gives an OD of >1.0 after 10min incubation at 22°C with ABTS substrate.

### Storage of protein A-HRP

The conjugate is shipped at ambient temperature and should be stored at 4°C. For very long-term storage small aliquots may be stored at -70°C, but avoid repeated cycles of freeze-thaw.

### Diluents

The conjugate may be diluted in any of the buffers commonly used in immunochemical procedures, typically either TBS or PBS pH 7.4 with 0.1% BSA. Tween 20 (0.05%) is sometimes used instead of BSA, or in addition to BSA. In most cases excellent results will be obtained with a simple buffer plus BSA combination.

### Notes

1. Lightning-Link™ is a proprietary conjugation system that permits a level of optimization of secondary reagents that is not feasible using standard conjugation methods.

### Appendix 1. Binding of protein A to immunoglobulins

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Pig	IgG	High
Mouse	IgG <sub>1</sub>	Medium/High*
Mouse	IgG <sub>2a</sub>	High
Mouse	IgG <sub>2b</sub>	High
Mouse	IgG <sub>3</sub>	High
Goat	IgG	Low/medium
Sheep	IgG	Low/medium
Rat	IgG	Low/medium
Mouse	IgM	Low/medium
Rabbit	IgM	Low

\*Mouse IgG<sub>1</sub> subclass shows higher affinity at pH 8.1. If you are detecting a mouse monoclonal antibody of IgG<sub>1</sub> subclass the signal strength may be enhanced by performing incubations at pH 8.1 rather than pH 7.4.

For data on other species/subclasses see Lindmark et al., J. Immunol. Methods 62 (1983) 1-13.

### FAQ

#### What dilution should I use?

This will depend on your particular application and on the HRP substrate that is employed. Popular HRP substrates include TMB, OPD and ABTS but the sensitivities vary over an order of magnitude, with TMB approximately 10x more sensitive than ABTS. For any new application try serial dilutions of conjugate and use the dilution that gives the highest signal to noise ratio with your preferred substrate.

For further information and related detection reagents see [www.innovabiosciences.com](http://www.innovabiosciences.com)