Amersham
Protein A, peroxidase-linked
Product Specification Sheet

Introduction

Product code

NA9120

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at 2–8°C. Do not freeze. Under these conditions, the product is stable for at least 6 months.

Expiry

See outer packaging.

Packaging

Protein A linked to Horseradish Peroxidase is supplied in Phosphate-Buffered Saline pH 7.5, containing 1% (w/v) Bovine Serum Albumin and an anti-microbial agent, in a total volume of 1 ml.

Preparation

This conjugate of peroxidase (from horseradish) and Protein A (1) (from Staphylococcus aureus) is prepared using the periodate method described by Nakane and co-workers (2).

Use

Protein A linked to Horseradish Peroxidase can be used to detect most classes of rabbit and human IgG and some classes of mouse and rat IgG (1). It therefore serves as a general purpose reagent for detecting antibodies and antibody-antigen complexes in enzyme immunoassay (3, 4). It is particularly useful to screen for protein A-binding monoclonal antibodies since protein A is often used in further purification and characterization of the antibodies.

Quality control

Plastic microtitration plates were coated with saturating amounts of human immunoglobulins in 0.1 M Carbonate/Bicarbonate buffer pH 9.5. Non-specific binding sites on the plates were blocked with 1% (w/v) Bovine Serum Albumin in Phosphate-Buffered Saline (PBS), pH 7.5, and the plates then washed with PBS containing 0.1% (v/v) Tween™20. The conjugate (NA9120) was diluted 1:5000 in PBS Tween plus 1% (w/v) BSA. 50 µl aliquots of the solution were added to the wells, and the plates incubated at 37°C for 3 hours. The plates were washed, 100 µl of substrate solution was added to each well, and the plates were incubated at 37°C. The reaction was stopped after 15 minutes and the absorbance at 405 nm determined. The absorbance was greater than 2.

References

1. SUROLIA, A. PAIND, D. and KHAN, M.I., Trends in Biochemical Sciences, 7, pp.74-76, 1982.
2. WILSON, M.B. and NAKANE, P.K., in Immunofluorescence and related staining techniques, edited by Knapp, W. et al., Elsevier (North Holland), pp.215-244, 1978.
3. GEE, A.P. and LANGONE, J.J., Analytical Biochemistry, 116, pp.524- 530, 1981.
4. YOLKEN, R.H. AND LEISTER, F.J., J. Immunol. Methods, 43, pp.209-218, 1981.

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