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Preparation of horseradish peroxidate (HRP) conjugated Peptostreptococcal protein-L by the periodate method.

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ABSTRACT

This reagent can be used in ELISA, Western blotting and Dot blot to detect antigens and antibodies. It is important in the immunodiagnosis of infectious diseases and other problems. Protein L binds to kappa light chains of immunoglobulins from many animal species including human, mouse, rat, chicken, hamster and pig [1].

1. Justiz-Vaillant AA, Akpaka PE, McFarlane-Anderson N, Smikle MF. Comparison of techniques of detecting immunoglobulin-binding protein reactivity to immunoglobulin produced by different avian and mammalian species. *West Indian Med J.* 2013;62(1):12-20.

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MATERIALS

NAME	CATALOG #	VENDOR
Ammonium Sulfate		P212121
Sodium periodate	SB0875.SIZE.100g	Bio Basic Inc.
sodium borohydride	452882	Sigma Aldrich
Horseradish Peroxidase (HRP) type IV	P8375-25KU	Sigma Aldrich
Protein-L from P. Magnus		

1 Horseradish peroxidase (500 µg in 50 µl NaCO3 , pH 9.6) is mixed with freshly made sodium periodate solution (1.71 mg/ml) followed by incubation in the dark for 2 h.

2 Mix 500 µg of protein-L (SpL) with an equal amount (500 micrograms) of a mix of horseradish peroxidase-sodium periodate.

3 The mixture is incubated for 3 hours at 4°C with gentle agitation.

4 Forty µl of freshly prepared NaBH4 solution (5 mg NaBH4 /ml 0.1 mM NaOH) is then added to the preparation.

5 The preparation is incubated for 90 min at 4°C in the dark with gentle agitation.

6 Cold 50% saturated ammonium sulphate solution (pH 7.4) is added drop by drop in the ratio 1:1 (v/v).

7 The mixture is then centrifuged for 25 min at 4°C and recover the pellet at the bottom of the tube.

8 The pellets is re-suspended in 200 µl of PBS pH=7.4 and dialysed against 1L of PBS for 24 h with 3 buffer changes.

9 An equal volume of glycerol is added to the dialysate followed by 100 µl of bovine serum albumin, BSA (20 mg/ ml).

10 The conjugate is then stored at -20°C until further used.

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