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Preparation of staphylococcal protein-A conjugated to horseradish peroxidase.

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We use this protocol and it's working

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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. I find this useful in the detection of anti-HIV antibodies by ELISA.

Materials

MATERIALS

Ammonium Sulfate **P212121**

Sodium periodate **Bio Basic Inc. Catalog #**SB0875.SIZE.100g

sodium borohydride **Sigma Aldrich Catalog #**452882

Horseradish Peroxidase (HRP) type IV **Sigma Aldrich Catalog #**P8375-25KU

Staphylococcal Protein-A **Sigma Aldrich**

Pipettes

20ml to 1000 ml glass

Scale

Incubator

Refrigerator

Freezer

Centrifuges

1 Horseradish peroxidase (500 µg in 50 µl $NaCO_3$, 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 staphylococcal protein-A (SpA) 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 $NaBH_4$ solution (5 mg $NaBH_4$ /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.