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Preparation of staphylococcal protein-A conjugated to horseradish peroxidate by the periodate method.

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Protocol status: Working
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 and other problems. 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

Safety warnings

Pay attention to all details as the times of reactions among the proteins involved in this preparation. It will prevent over-oxidation. The average time of preparation is 18 hours.

Before start

All reagents but specially the enzyme and more importantly the sodium periodate solution has to be prepared freshly before mixing it with the enzyme.

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.

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