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Preparation of a protein-AG conjugated to horseradish peroxidase by the periodate method.

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We use this protocol and it's working

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Abstract

A recombinant protein that combines the IgG-binding domains of SpA and SpG was developed, and labelled to horseradish peroxidase. It was used as universal conjugate in ELISA for the assessment of antibodies against Brucella spp in cattle, sheep, dogs, goats and pigs. It was reported that similar results as the one shown using the chimeric protein AG were obtained when murine monoclonal antibody-enzyme conjugates were used [1,2].

References

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.

2. Justiz-Vaillant AA, McFarlane-Anderson N, and Smikle M. “Bacterial Immunoglobulin (Ig)-Receptors: Past and Present Perspectives.” American Journal of Microbiological Research, vol. 5, no. 2 (2017): 44-50. doi: 10.12691/ajmr-5-2-4.

Guidelines

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

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**
- Streptococcal protein G by 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. On the other hand mix 500 µg of streptococcal protein-G (SpG) with an equal amount (500 micrograms) of the mix of horseradish peroxidase-sodium periodate.

3 The two mixtures are incubated separately 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 separately to the preparations, which are centrifuge (13,000rpm., 10 minutes at RT). Add to each preparation cold saturated amonium sulphate solution and centrifuge again (10000rpm, 25 minutes at 4°C).

5 Now mix the SpA-HRP preparation with SpG-HRP and incubate the mixture for 90 min at 4°C in the dark with gentle agitation.

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

7 The pellet (SpAG-HRP) is re-suspended in 500 µl of PBS pH=7.4 and dialysed against 1L of PBS for 24 h with 3 buffer changes.

8 An equal volume of glycerol is added to the dialysate followed by 200 µl of bovine serum albumin, BSA (20 mg/ ml).

9 The SpAG-HRP conjugate is then stored at -20°C until further used.