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 We use this protocol and it's working

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

Angel A Justiz-Vaillant¹

¹University of the West Indies St. Augustine

University of the West Indies

angel.vaillant@sta.uwi.edu

Angel A Justiz-Vaillant

University of the West Indies St. Augustine

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 **Contributed by users**

Pipettes

20ml to 1000 ml glass

Scale

Incubator

Refrigerator

Freezer

Centrifuges

- 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 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 NaBH₄ solution (5 mg NaBH₄ /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 ammonium 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.