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Protocol of preparation of a protein-LAG conjugated to horseradish peroxidase by the periodate method.

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Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

University of the West In... angel.vaillant@sta.uwi.e...

Angel A Justiz-Vaillant
University of the West Indies St. Augustine

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We use this protocol and it's working

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Abstract

Peroxidase conjugated to proteins L, A and G (SpLAG-HRP) is a new development in the field of biochemistry. This versatile protein binds to more 500 species of animal IgGs. It makes it suitable for the study of zoonosis and the assessment of the antibody production to infectious microorganisms. This immunomarker is not available in a recombinant form as their counterpart SpLA-HRP, SpLG-HRP and SpAG-HRP. It can be used in ELISA, immunohistochemistry, Western blot analysis, Dot blot, and RIA [1,2].

References

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2. Vaillant AJ, McFarlane-Andersonv N, Wisdom B, Mohammed W, Vuma S, et al. (2013) Immunoglobulin-binding Bacterial Proteins (IBP) Conjugates and their Reactivity with Immunoglobulin in Enzyme-Linked Immunosorbent Assays (ELISA). J Anal Bioanal Tech 4: 175. doi:10.4172/2155-9872.1000175.

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 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**452882

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

Staphylococcal Protein-A **Merck MilliporeSigma (Sigma-Aldrich)**

Protein-L from P. Magnus

Streptococcal protein G by Sigma Aldrich

Pipettes
20ml to 1000 ml glass
Scale
Incubator
Refrigerator
Freezer
Centrifuges

Troubleshooting

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

3 The three 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 to each mixture and then, the preparations are centrifuged (13,000rpm., 10 minutes at RT). Add to each preparation cold saturated amonium sulphate solution and centrifuge them again (10000rpm, 25 minutes at 4°C).

5 Mix the 3 preparations and incubate 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 (SpLAG-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 SpLAG-HRP conjugate is then stored at -20°C until further used.