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Aug 12, 2020

# Protocol of preparation of a Protein-LAG conjugated to horseradish peroxidase-labeled immunoglobulin Y (IgY-HRP).

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1 Works for me dx.doi.org/10.17504/protocols.io.bjk8kkzw

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ABSTRACT

Peroxidase-labeled anti-IgY conjugated to proteins L, A and G (SpLAG-anti-IgY-HRP) is a new development in the field of biochemistry. This versatile protein binds to more 700 species of animal IgGs including birds, laboratory animals, farm animals, wild animals, pets, and many others. This conjugate can be used in the study of zoonosis and the assessment of the antibody production for the study of the animal immune system. 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 [1-4].

References

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DOI

dx.doi.org/10.17504/protocols.io.bjk8kkzw

PROTOCOL CITATION

Angel A Justiz-Vaillant 2020. Protocol of preparation of a Protein-LAG conjugated to horseradish peroxidase-labeled immunoglobulin Y (IgY-HRP). . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bjk8kkzw

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CREATED

Aug 11, 2020



**Citation:** Angel A Justiz-Vaillant (08/12/2020). Protocol of preparation of a Protein-LAG conjugated to horseradish peroxidase-labeled immunoglobulin Y (IgY-HRP).ÃÂ . https://dx.doi.org/10.17504/protocols.io.bjk8kkzw

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LAST MODIFIED

Aug 12, 2020

PROTOCOL INTEGER ID

40320

GUIDELINES

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

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Ammonium Sulfate | | P212121 |
| Anti-Chicken IgY, HRP Conjugate, 300ul | G1351 | Promega |
| Sodium periodate | SB0875.SIZE.100g | Bio Basic Inc. |
| sodium borohydride | 452882 | Sigma Aldrich |
| Staphylococcal Protein-A | | Sigma Aldrich |
| Protein-L from P. Magnus | | |
| Streptococcal protein G by Sigma Aldrich | | |

1 Horseradish peroxidase-labeled IgY (Promega) (500 µl in 50 µl $NaCO3$ , pH 9.6) is mixed with freshly made sodium periodate solution (1.71 mg/ml). Then the mixture is incubated 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. These mixtures are incubated for 2 hours at 4°C with gentle agitation.

3 Forty µl of freshly prepared $NaBH4$ solution (5 mg $NaBH4$ /ml 0.1 mM NaOH) is then added to each mixture, including to the anti-IgY-HRP.

4 Then, the preparations are centrifuged (13,000 rpm., 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 bacterial antigen preparations with the anti-IgY-HRP and incubate it 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-anti-IgY-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).



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



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