Upload image

Aug 15, 2020

Universal sandwich ELISA for investigating the binding of avian and mammalian immunoglobulins to Streptococcal protein-G (SpG) using a peroxidase-labeled Protein LAG conjugate (SpLAG-HRP).

Angel A Justiz-Vaillant[1]

[1]University of the West Indies St. Augustine

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

University of the West Indies angel.vaillant@sta.uwi.edu

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

DOI

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

PROTOCOL CITATION

Angel A Justiz-Vaillant 2020. Universal sandwich ELISA for investigating the binding of avian and mammalian immunoglobulins to Streptococcal protein-G (SpG) using a peroxidase-labeled Protein LAG conjugate (SpLAG-HRP).. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bjsfknbn

CREATED

Aug 15, 2020

LAST MODIFIED

Aug 15, 2020

PROTOCOL INTEGER ID

40487

MATERIALS

NAME	CATALOG #	VENDOR
Anti-Chicken IgY, HRP Conjugate, 300ul	G1351	Promega
Streptococcal protein G by Sigma Aldrich		

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the

1 This ELISA is used to study the interaction of streptococcal protein-G (SpG) with different avian and mammalian immunoglobulins.

2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of SpG in carbonate-bicarbonate buffer pH 9.6.

3 Then plate is treated with bovine serum albumin solution and washed 4X with PBS-Tween.

4 50 µl of avian egg yolk, egg white (1:8 dilutions) or 50 µl of sera or 50 µl of mammalian IgG (1mg/ml) is added to the well and incubated for 1.30h at room temperature and the microplate is then rewashed 4X with PBS-Tween.

5 Then 50 µl of peroxidase-labeled-Protein-LAG (SpLAG-HRP) conjugate diluted 1:15000 in PBS-non-fat milk is added to each well and incubated for 1.30h at RT. The plate is washed 4X with PBS-Tween.

6 Pipette 50 µl of TMB (Sigma-Aldrich) to each well.

7 The reaction is stopped with 50 µl of 3M H_2SO_4 solution.

8 The plate is visually assessed for the development of colour and read in a microplate reader at 450 nm.

9 A cut-off point should be calculated as the mean of the optical density of negative controls x 3. The higher the OD value the higher will be the affinity of SpG to immunoglobulins.

Citation: Angel A Justiz-Vaillant (08/15/2020). Universal sandwich ELISA for investigating the binding of avian and mammalian immunoglobulins to Streptococcal protein-G (SpG) using a peroxidase-labeled Protein LAG conjugate (SpLAG-HRP).. https://dx.doi.org/10.17504/protocols.io.bjsfknbn