Aug 15, 2020

Immunoblot analyses for investigating SpLG-binding to purified mammalian and avian immunoglobulins.

DOI
dx.doi.org/10.17504/protocols.io.bjrukm6w

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

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.bjrukm6w

Protocol Citation: Angel A Justiz-Vaillant 2020. Immunoblot analyses for investigating SpLG-binding to purified mammalian and avian immunoglobulins.. **protocols.io https://dx.doi.org/10.17504/protocols.io.bjrukm6w**

Protocol status: Working
We use this protocol and it's working

Created: August 15, 2020

Last Modified: August 15, 2020

Protocol Integer ID: 40468

1 Aliquots of egg yolks, sera or 2 µg/µl of purified immunoglobulins from birds, laboratory, wild, farm animals and pets are applied to the gels of SDS-PAGE as described elsewhere.

2 Gels are transferred to nitrocellulose membranes (Immobilon-Nc, pore size 0.45 µm, Sigma-Aldrich Co, St Louis, Missouri) during 75 minutes at 40 mAmps using a semi-dry electroblotter, HEP-1 Model, Owl Scientific Inc.

3 The running buffer contains 25 mM Tris, 192 mM glycine pH 8.3 and 20% methanol.

4 The nitrocellulose membranes are blocked overnight in 10% non-fat skim milk in PBS with 0.05% Tween-20 pH 7.4 and then washed 4x, 10 minutes with PBS-Tween 20.

5 Recombinant protein LG or a mixture of SpL and SpG at a concentration of 5 µg/ml is added to the membranes.

6 After that there is an incubation period at 4°C overnight.

7 The nitrocellulose membranes were washed as above.

8 A secondary antibody (rabbit anti-chicken IgY horseradish peroxidase, Sigma Aldrich) is added at a 1:15 000 dilution.

9 It is incubated for one hour at room temperature and washed as above.

10 Tetramethyl-benzidine (TMB) solution is added to the nitrocellulose membranes, which are then incubated in the dark for seven minutes. Then, the membranes are shaken gently and rinsed thoroughly in de-ionized water to stop the blotting process and are left to dry.

11 Alternatively, Ig samples are transferred to nitrocellulose membranes and directly probed using SpLG-HRP (diluted 1:5000) and TMB (this system was mainly used for detecting avian Igs).