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Universal Immunoblot analysis for investigating Protein-AG (SpAG)-binding to avian and mammalian immunoglobulins.

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

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ABSTRACT

A protein that combines the binding capacity of SpA and SpG is not comercially available. It can be easy created in the laboratory by combining these two immunoglobulin-binding proteins to horseradish peroxidase by the periodate method [1]. However, a mixture of SpA and SpG could have the same effect as universal reagent in immunodetection.

1. 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

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1 Aliquots of egg yolks, animal 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 71 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 A mixture of SpA and SpG at a concentration of 5 µg/ml is added to the membranes. A recombinant Protein-AG (SpAG) could be used instead.

6 After that there is an incubation period of 12 hours at 4°C. It may be an overnight incubation period.

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 SpAG-HRP (diluted 1:5000) and then adding TMB (this system was mainly used for detecting avian Igs).

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