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Enzyme linked immunosorbent assay for investigating the binding of chemically prepared protein-LAG-anti-IgY (SpLAG-anti-IgY) to avian and mammalian immunoglobulins.

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ABSTRACT

This SpLAG-anti-IgY ELISA can be used to detect specific antibodies in various animal species including human, goat, donkey, mouse, rat, dog, cow, horse, ostrich, duck, pigeon, bantam hen, rabbit, chicken, monkey, pig, hamster, and many birds, wild and zoo animals species [1].

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 This ELISA is used to study the interaction of protein-LAG-anti-IgY (SpLAG-anti-IgY) with different immunoglobulin preparations from avian and mammalian species.

2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of a mixture of SpL, SpA, SpG and anti-IgY

(equal concentration of each protein) 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 animal serum (1 mg/ml) is added and incubated for 1h at room temperature and the microplate is rewashed 4X with PBS-Tween.

5 Then 50 µl of peroxidase-labeled SpLAG-anti-IgY conjugate diluted 1:5000 in PBS-non-fat milk is added to each well and incubated for 1h at RT. The plate is washed 4X with PBS-Tween.

6 50 µl of 4 mg/ml o-phenylenediamine solution (OPD) is added and the plate is incubated 15 minutes at RT in the dark.

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 492 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 SpLAG-anti-IgY to avian and mammalian immunoglobulins.

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