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## Enzyme linked immunosorbent assay for investigating the binding of Protein-AG (SpAG) to immunoglobulins.

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**We use this protocol and it's working**

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## Abstract

This SpAG ELISA can be used to detect specific antibodies in various animal species including human, goat, donkey, mouse, rat, dog, cow, horse, rabbit, chicken, monkey, pig and hamster [1].

1. Kelly PJ, Tagwira M, Matthewman L, Mason PR, Wright EP. Reactions of sera from laboratory, domestic and wild animals in Africa with protein A and a recombinant chimeric protein AG. *Comp Immunol Microbiol Infect Dis*. 1993;16(4):299-305. doi:10.1016/0147-9571(93)90159-3

- 1 This ELISA is used to study the interaction of recombinant protein-AG (SpAG) with different immunoglobulin preparations.
- 2 The 96 well microtitre plate is coated overnight at 4°C with 2 µg/µl per well of SpAG 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 SpAG 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<sub>2</sub>SO<sub>4</sub> 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 SpAG to immunoglobulin G.