AUG 13, 2020

OPEN ACCESS

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

Protocol Citation: Angel A Justiz-Vaillant, Norma McFarlane-Anderson 2020. Enzyme linked immunosorbent assay for investigating the binding of Protein-AG (SpAG) to immunoglobulins..
protocols.io
<https://dx.doi.org/10.17504/protocols.io.bjppkmmn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 13, 2020

Last Modified: Aug 13, 2020

PROTOCOL integer ID:
 40399

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

Angel A Justiz-Vaillant¹, Norma McFarlane-Anderson²

¹University of the West Indies St. Augustine;

²University of West Indies. Mona Campus

University of the West Indies

angel.vaillant@sta.uwi.edu

Angel A Justiz-Vaillant

University of the West Indies St. Augustine

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 H₂SO₄ 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.