AUG 13, 2020

🌐 Enzyme linked immunosorbent assay for investigating the immunoglobulin-binding bacterial protein (IBBP) to avian egg yolk antibodies.

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OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bjnzkmf6

Protocol Citation: Angel A Justiz-Vaillant 2020. Enzyme linked immunosorbent assay for investigating the immunoglobulin-binding bacterial protein (IBBP) to avian egg yolk antibodies..

protocols.io

<https://dx.doi.org/10.17504/protocols.io.bjnzkmf6>

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

Created: Aug 13, 2020

Last Modified: Aug 13, 2020

PROTOCOL integer ID:
40377

1 This ELISA is used to study the interaction of proteins A, L and LA with different avian IgY

preparations.

- 2 The 96 well microtitre plate is coated overnight at 4°C with 1 µl/mg per well of unlabelled SpL, SpA or SpLA 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 different IgY preparations (1 mg/ml) are added and incubated for 1h at room temperature and washed.
- 5 Then 50 µl of rabbit anti-chicken IgG-HRP conjugate diluted 1:15000 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 plates are incubated 15 minutes at RT.
- 7 The reaction is stopped with 50 µl of 3M H₂SO₄ solution.
- 8 The plate is visually assessed for the development of colour.
- 9 The plate is read in a microplate reader at 492 nm.

10 A cut-off point is calculated.