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Copy of Enzyme-linked immunosorbent assay (ELISA) for studying the presence of anti-Salmonella antibody in layer hen's egg yolks.

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1 Works for me dx.doi.org/10.17504/protocols.io.bj56kq9e

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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) for studying the presence of anti-*Salmonella* antibody in layer hens was a reproducible and feseable test used to meassure IgY development after vaccination.

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GUIDELINES

After adding the substrate, keep the ELISA plate in a very dark place to get reproducible results.

MATERIALS

NAME	CATALOG #	VENDOR
Anti-Chicken IgY, HRP Conjugate, 300ul	G1351	Promega
LPS	L3129	Sigma-aldrich
eBioscience™ TMB Solution (1X)	00-4201-56	Thermo Fisher
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, U 96 well plate, Non-Treated, clear, with lid, Sterile	268200	Thermo Fisher
ELISA Coating Buffer (5X)	421701	BioLegend

1 U-shaped bottom's ninety-six well polystyrene microplate purchased at Sigma-Aldrich, St. Louis USA was incubated

with (2 µg/well) of the LPS (Sigma –Aldrich) from Salmonella Typhimurium in coating buffer (overnight at 4 ºC.)

2 The microtiter plates was washed four times, with 10 % PBS-Tween-20.

3 The microplate was blocked with 3% non-fat milk in PBS (25 µl/well).

4 The microplate was incubated 1 hr at RT .

5 The microplate was washed four times.

6 Then a 50 µl aliquot of the egg yolk (Ig)Y solutions in a concentration of 1.25 mg/ml was added in triplicate. The IgY concentration was assessed by ELISA and sample were titrated with sample buffer until it got the expected IgY concentration.

7 After incubating for one hour at RT, the microplate was washed four times.

8 Fifty (50 µl) of the anti-IgY-HRP conjugate (Sigma-Aldrich) diluted to 1:30000 with conjugate diluent was added into each well.

9 The microplate was incubated for 1 hr at RT.

10 Then, the microplate was washed four times.

11 Fifty (50 µl) of tetramethylbenzidine (TMB, Sigma-Aldrich) was added into each well.

12 The microplate was further incubated for 15 minutes in the dark.

13 Fifty (50 µl) 3M HCl was added to the microplate for stopping the reaction.

14 After that, reaction color development was measured with a microplate reader (Synergy™ Neo Hybrid Multi-Mode

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Microplate Reader).

15 The cut-off point was an OD of 0.51, and it was calculated from the XOD of the negative x 2 SD. This ELISA tested triplicates of a total of 90 IgY preparations.

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