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

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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.**

## DOI

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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
<a href="#">Anti-Chicken IgY, HRP Conjugate, 300ul</a>	G1351	<a href="#">Promega</a>
<a href="#">LPS</a>	L3129	<a href="#">Sigma-aldrich</a>
<a href="#">eBioscience&amp;trade; TMB Solution (1X)</a>	00-4201-56	<a href="#">Thermo Fisher</a>
<a href="#">Nunc&amp;trade; 96-Well Polystyrene Round Bottom Microwell Plates, U 96 well plate, Non-Treated, clear, with lid, Sterile</a>	268200	<a href="#">Thermo Fisher</a>
<a href="#">ELISA Coating Buffer (5X)</a>	421701	<a href="#">BioLegend</a>

- 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 °C.)
- 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 Microplate Reader).
- 15 The cut-off point was an OD of 0.51, and it was calculated from the XOD of the negative control times 3. This ELISA tested triplicates of a total of 90 IgY preparations.