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Enzyme-linked immunosorbent assay (ELISA) for detection of anti-HIV antibodies (ELISA) developed in various animal species.

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1 Works for me dx.doi.org/10.17504/protocols.io.bjmzkk76

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ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) for studying the presence of anti-HIV antibodies in layer hens, rats and cats was a reproducible and feseable test. The presence of the anti-HIV antibodies was further confirmed by dot blot analysis.

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MATERIALS

NAME	CATALOG #	VENDOR
3,3',5,5'-Tetramethylbenzidine	54827-17-7	Sigma Aldrich
ELISA Diluent 2 x 120 mL	1916	Stemcell Technologies
Nunc™ 96-Well Polystyrene Round Bottom Microwell Plates, V 96 well plate, Non-Treated, clear, without lid, Sterile	260210	Thermo Fisher
ELISA Blocker Blocking Buffer	N502	Thermo Fisher
Coating Buffer	421701	BioLegend
Protein LAG-anti-IgY-peroxidase (homemade)		

SAFETY WARNINGS

The risk associated with a particular microbe can be consulted in this website of the CDC

(http://www.cdc.gov/biosafety/publications/BiologicalRiskAssessmentWorksheet.pdf)

DISCLAIMER:

1 The 96 well polystyrene microplates (U-shaped bottom) were coated with 50 ng of the synthetic gp-120 peptide in coating buffer overnight at 4°C.

2 The microplate is washed 4X (PBS-Tween-20) and blocked with 3% non-fat milk in PBS, 25 µl/well, 1h, RT.

3 The microplate is washed 4X (PBS-Tween-20) and triplicates of 25 µl of IgY (1 mg/ml), 25 µl of sera from cats or rats diluted 1:16 in PBS-non fat milk is added.

4 After incubation for 2 h at RT the microplate is washed 4X and 25 µl of a universal conjugate SpLAG-anti-IgY-HRP (comprised of peroxidase labelled-IgY chemically coupled to protein A, protein G and protein L) diluted 1:1000 was added and the plate incubated for a further 1 h at RT.

5 Then, the microplate is washed 4X and after that 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co., St Louis, MO, USA) solution (50 µl) is added to each well.

6 After a further incubation of 15 min in the dark, the reaction is stopped when positive controls are coloured and blanks and negative controls do not.

7 Positive controls are from a positive human serum for anti-HIV antibodies, negative controls are from a turtle serum (Sigma Chemical Co., St Louis, MO, USA) and blanks 0.9% normal saline solution.

8 Cut-off point is calculated as the mean of negative controls multiplied by four.

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