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Indirect ELISA for detection of anti-HIV antibodies in cats immunized with a HIV gp120 peptide.

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- 1 The ELISA describes detection of antibodies against HIV proteins. It is an ELISA with a modification. The plate is coated with HIV peptides, fragment 254-274 of gp120.
- 2 The modification is that triplicates of 1/16 dilutions of cat sera in PBS non-fat milk are added to the micro-wells.
- 3 After incubation for 90 min at RT the microplate is washed 4X using PBS-Tween 20.
- 4 Then, 25 µl of recombinant protein LA-HRP diluted 1:1000 (Sigma-Aldrich) is added to the assay.
- 5 The microplate is incubated for 1h at RT and then, it is washed four times.

- 6 Tetramethylbenzidine (TMB) solution (50 μ l) is added to each well. .
- 7 After a further incubation of 15 min in the dark, the reaction is stopped.
- 8 The microplate is read at 450 nm in a microplate reader at 450 nm. Geometric mean antibody titer is calculated using Perkins method.