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

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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.

1. The modification is that triplicates of 1/16 dilutions of cat sera in PBS non-fat milk are added to the micro-wells.

2. After incubation for 90 min at RT the microplate is washed 4X using PBS-Tween 20.

3. Then, 25 µl of recombinant protein LA-HRP diluted 1:1000 (Sigma-Aldrich) is added to the assay.

4. The microplate is incubated for 1h at RT and then, it is washed four times.
Tetramethylbenzidine (TMB) solution (50 μl) is added to each well.

After a further incubation of 15 min in the dark, the reaction is stopped.

The microplate is read at 450 nm in a microplate reader at 450 nm. Geometric mean antibody titer is calculated using Perkins method.