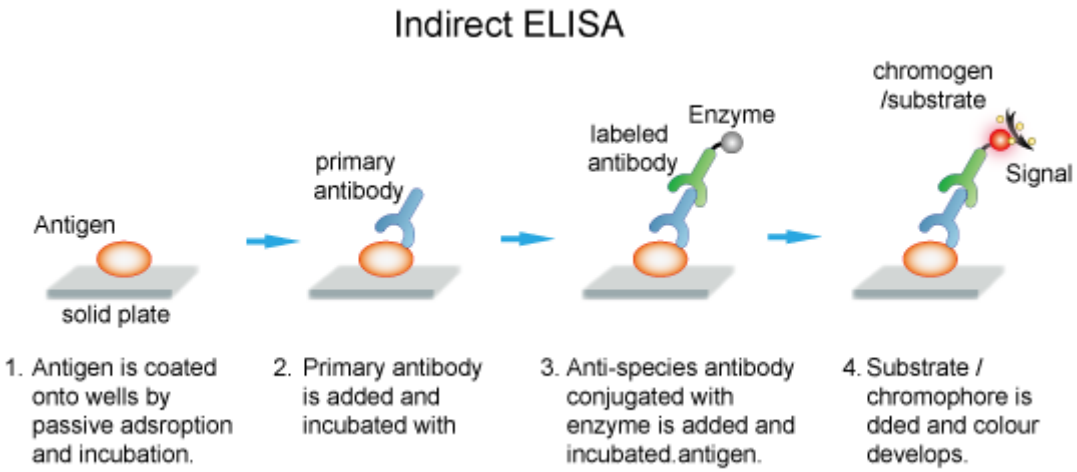
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The Enzyme-Linked Immunosorbent Assay (ELISA) is an immunological technique used in biochemistry for the detection and quantification of antigens or antibodies. Depending on the intended purpose, different methods can be followed, all based on the ability to detect the antigen-antibody complex using a second antibody capable of binding the first and conjugated with a particular enzyme (usually alkaline phosphatase or horseradish peroxidase) that catalysis a color reaction with the addition of a suitable substrate. The development of color is indicative of the presence of the antigen or antibody to be assayed and the intensity of the coloration can be measured using a spectrophotometer.



Indirect ELISA

The indirect detection method adds a labeled secondary antibody for detection on the basis of direct ELISA and it is the most popular ELISA format. Antigen is passively attached to wells by incubation. After washing, antibodies specific for the antigen are incubated with the antigen. Wells are washed and all bound antibodies are detected by the addition of anti-species antibodies covalently linked to an enzyme. Such antibodies are specific for the species in which the first antibody added were produced. After incubation and washing, the test is developed and can be read as described in figure.

PRINCIPLE: Indirect absorbed ELISA for detection of anti-*Mycobacterium avium* subsp. *paratuberculosis* antibodies in plasma.

MATERIALS PROVIDED

- MAP antigen-coated microplate (96 wells)
- Sample
- Conjugate (Anti-ruminant IgG-HRP, concentrated 10X)
- Positive control 12X
- Negative control 12X
- Substrate solution (3,3',5,5'-Tetramethylbenzidine TMB/H_2O_2)
- Stop solution (0.5 M H_2SO_4)

- Wash buffer (concentrated 20X)
- Dilution buffer 6
- Dilution buffer 3

PROCEDURE

1. SAMPLE and REAGENTS PREPARATION

In a 96-well pre-dilution microplate, prepare the samples and controls:

- Bring all reagents to room temperature (21 ± 5 °C)
- Dilute samples 2X (using dilution buffer 6)
- Dilute, using dilution buffer, positive and negative controls (V_f = 120)
- Cover plate and incubate for 20 minutes at 21 °C ± 5 °C
- Transfer 100 µL of the controls and samples to the ELISA microplate
- Cover plate and incubate for 20 minutes at 21 °C ± 5 °C (RT)

2. WASHING

- Empty wells and wash 3 times with 300 µL wash buffer
- Tap plate on absorbent paper to remove residual liquid

3. CONJUGATE ADDITION

- Dilute conjugate in conjugate diluent (Dilution buffer 3)
- Add 100 µL conjugate to each well
- Cover plate and incubate 30 minutes at 21 °C ± 5°C (RT)

4. WASHING

- Repeat washing step (3 times with 300 µL wash buffer)

5. SUBSTRATE REACTION

- Add 100 µL substrate solution to each well
- Incubate 15 minutes at RT in darkness
- Monitor color development

6. STOP REACTION

- Add 100 µL stop solution to each well
- Mix gently

7. READING

- Read absorbance at 450 nm within [X] minutes

CALCULATIONS

Sample-to-Positive (S/P) Ratio:

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S/P % = [(OD Sample - OD Negative Control) / (OD Positive Control - OD Negative Control)] × 100
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Interpretation:

Result	**S/P %**
Negative	S/P < 30%
Positive	S/P ≥ 30%

QUALITY CONTROL

Acceptance Criteria:

- OD Positive Control ≥ 0.350
- OD Positive Control / OD Negative Control ≥ 3