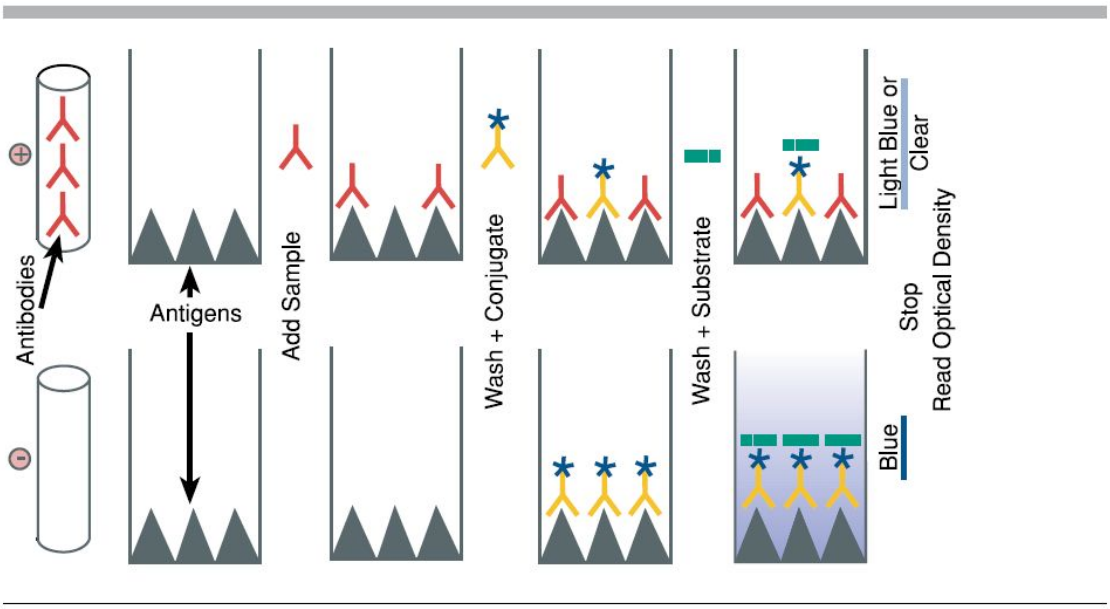
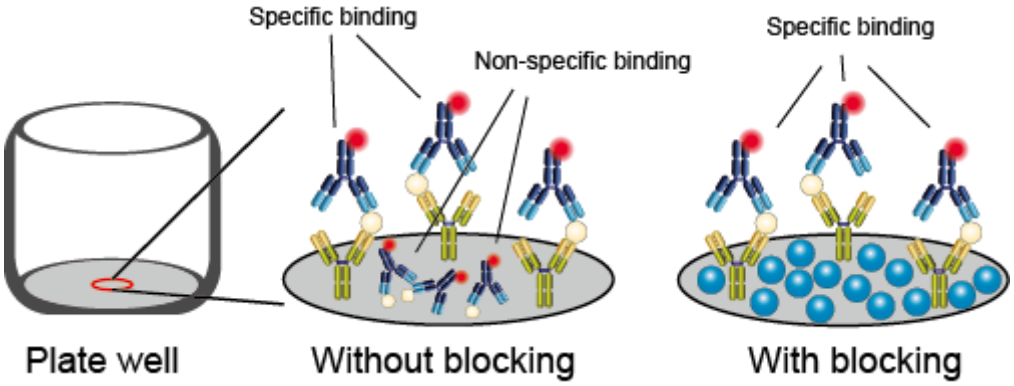
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

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.





cELISA

Components provided by the kit

- 96-well coated microplate
- Wash Buffer: 0.05% Tween-20 in PBS, pH 7.2, 7.4
- Dilution buffer: 1% BSA in PBS, pH 7.2, 7.4
- Positive control serum lyophilized
- Negative control serum lyophilized
- Monoclonal antibody peroxidase conjugate 100x
- Substrate solution: 3,3’,5,5’-Tetramethylbenzidine

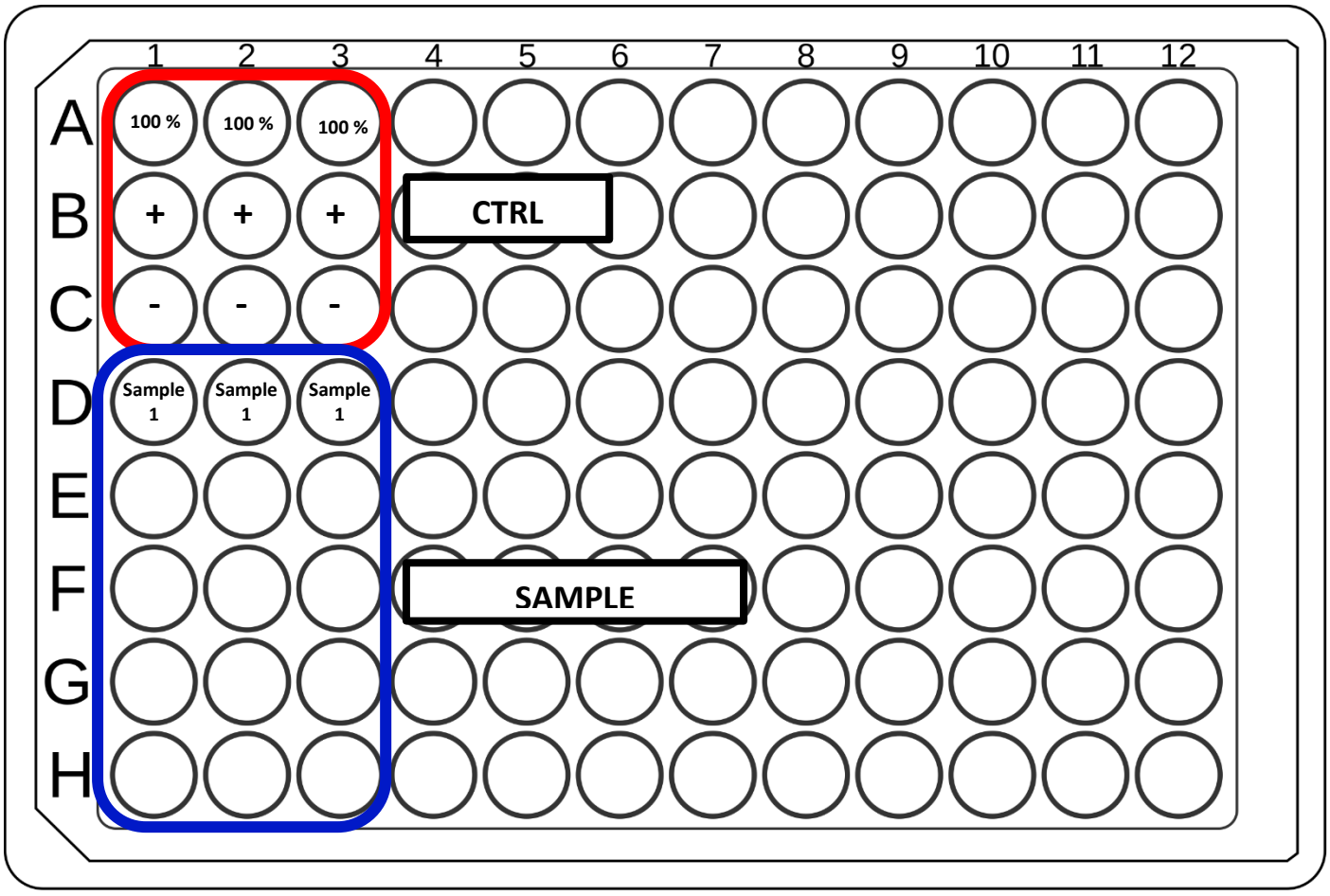
- Stop solution: 2n H_2SO_4

REAGENT PREPARATION

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Reconstitute controls with 1ml of H_2O deionized.

		Antibody peroxidase conjugate	3,3',5,5'-Tetramethylbenzidine
100%	50 µl Buffer E	50 µl	100 µl
+	50 µl Positive CTRL	50 µl	100 µl
-	50 µl Negative CTRL	50 µl	100 µl
Sample 1	50 µl serum	50 µl	100 µl
Sample 2	50 µl serum	50 µl	100 µl
Sample 3	50 µl serum	50 µl	100 µl



Assay procedure

Dispense 50 µl/well of Dilution buffer, positive control, negative control and incubate plate 2 hour at room temperature.

1. Wash plate 3 times with wash buffer, 200 µl/well

2. Add 50 µL/well of the Antibody peroxidase conjugate, diluted 1:100 in Dilution buffer Cover plate and incubate 1 h at room temperature.

3. Wash plate 3 times with wash buffer, 200 µl/well

4. Add 100 µL of Substrate solution to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.

5. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

6. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.
7. **Data analysis**

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

In a competitive ELISA, the plate is coated with the target antigen. When a serum sample is added, any specific antibodies present in the sample will bind to the antigen. Next, a labeled secondary antibody is added. This antibody is designed to bind the antigen as well, but it competes with the antibodies in the sample. If the sample contains high levels of antibodies, they will block the antigen-binding sites, preventing the labeled antibody from attaching. This results in a low signal (low color development). If no or few antibodies are present in the sample, the labeled antibody will bind to the antigen freely, leading to a high signal (strong color development).

Since this test is qualitative, the results are usually expressed as positive or negative based on a cutoff value. The absorbance (optical density, OD) of each sample is compared to a negative control (serum without antibodies).

- Positive result: Low OD, indicating the presence of antibodies in the sample.
- Negative result: High OD, indicating the absence of specific antibodies.

A percentage inhibition (PI%) is often calculated:

$$PI\% = \left(OD\,\frac{sample}{100\%\ reaction}\right) * 100$$

Where 100% reaction is represented by the conjugate control, meaning the well where the enzyme-conjugated antibody binds completely to the antigen without competition from specific antibodies in the serum.