Basic Immunology Practice – ELISA

In an indirect ELISA, the antigen to be tested is already bound to the surface of the plate. The sample is then added, which contains the specific antibody of interest.
Detection is carried out using a secondary antibody conjugated to an enzyme (peroxidase, PO). When the substrate (TMB) is added, a colour reaction occurs. The intensity of the colour is directly proportional to the amount of antibody present in the sample.

Required materials and equipment

- 96-well microtiter plate pre-coated with antigen, filled with gelatine blocking solution (300 µl/well)
- Wash buffer
- Samples (dilution factors indicated in parentheses) and control solutions in labelled tubes (100 µl/tube):

1. (1x)	**2.** (5x)	**3.** (25x)	**4.** (50x)	**5.** (100x)	**6.** (200x)	neg. crtl. (wash buffer)	poz. ctrl. (IgG 1:1000)

- Conjugate: anti-mouse IgG peroxidase (PO), 1:10,000
- Substrate: TMB (3,3',5,5'-tetramethylbenzidine)
- STOP solution

Procedure

1. Remove the gelatine blocking solution from the plate. Pour it off with a firm motion. Turn the plate upside down and tap it onto a clean paper towel.

2. Washing (3x)

- Add **300 µl** of wash buffer to each well. Pour it off with a firm motion. Turn the plate upside down and tap it onto a clean paper towel. Repeat this step two times. Follow the same procedure for all subsequent washing steps.

3. First incubation (antibody binding)

- After the third wash, add **100 µl** of each sample and the positive and negative controls into the wells.
- Add the samples in the following order:

1.	1x
2.	5x
3.	25x
4.	50x
5.	100x
6.	200x
7.	neg. crtl. (wash buffer)
8.	poz. ctrl. (IgG 1:1000)

- **Incubate** at room temperature for **35 minutes**.

4. Washing 3x

- Repeat the washing step 3 times as described above (step 2).

5. Incubation with the secondary antibody

- Add **100 µ**l of anti-mouse IgG PO conjugate to each well.
- IIncubate at room temperature for **35 minutes**.

6. Washing 3x

- Repeat the washing step 3 times as described above (step 2).

7. Colour development and stopping the reaction

- **Substrate addition**: Add **100 µl of TMB** solution to each well. Wait until a blue colour develops.
- **Stopping**: Add **50 µl of STOP** solution. The blue colour will change to yellow.