ELISA test protocol

Overview of the tubes:

color	contents	labelling	quantity
green	antigene	"AG"	800 µl
orange	secondary antibody	"sAB"	800 µl
brown	substrate	"SUB"	800 µl
violet	primary antibody	"pAB"	800 µl
blue	washing buffer	"WB"	1600 µl
yellow	Still empty!	"?"	- -

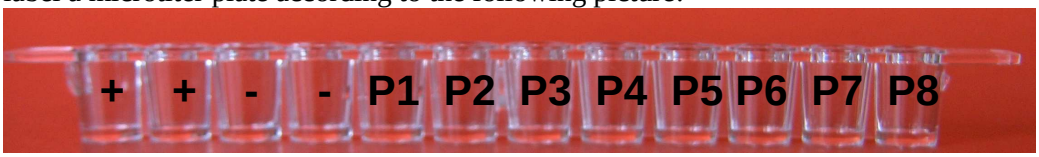
1. Preparation of samples P1 – P8 (they correspond the serum samples to be tested)

- label the 8 yellow tubes with your group number!
- pipet 60 µl **primary antibody solution** (pAB, positive result of the test)
  or
  60 µl **washing buffer** (WB, negative result of the test)
  into each of the 8 yellow tubes! Write down your set-ups (P1 – P8 are serum samples)!
- distribute 1 tube to each of the eight teams!

2. Execution of the test

2.1 Antigen binding on microtiter plate

- label a microtiter plate according to the following picture!



- pipet 50 µl antigen solution (AG, green tube) into each of the 12 wells!
- incubate for 5 minutes!

2.2 Washing the microtiter plates

- tap the plates on tissues!
- pipet 250 µl washing buffer into each well!

! Attention: During pipeting the washing buffer should not spill over into other wells!

- tap the plate on new tissues!

2.3 Preparation of controls and samples

- positive control: pipet 50 µl primary antibodies (pAB, violet tube) into 2 with " + " labelled wells!
- negative control: pipet 50 µl washing buffer (WB, blue tube) into 2 with " - " labelled wells!
- samples 1 – 8: pipet 50 µl "serum samples of the test person" P1 – P8 (yellow tubes) into the corresponding labelled 8 wells!
- incubate for 5 minutes!

2.4 Washing the microtiter plates (see 2.2)!

2.5 Addition of secondary antibody

- pipet 50 µl secondary antibodies (sAB, orange tube) into each of the 12 wells!
- incubate for 5 minutes!

2.6 Wash the microtiter plates twice (see 2.2)!

2.7 Addition of substrate

- pipet 50 µl substrate (SUB, brown tube) into each of the 12 wells!
- incubate for 5 minutes!

3. Evaluation:

Positive samples are blue-colored, negative samples stay colorless (controls).
Check your test results (P1 – P8) in the team!