ELISA

The human SARS-CoV-2 IgG ELISA is an indirect ELISA which is based on the antibody/antigen interactions. This ELISA allows the detection of SARS-CoV-2 Spike, RBD or Nucleocapsid specific IgG antibodies in human serum samples, and it can be adapted to animal samples as well.

The SARS-CoV-2 pre-fusion spike, RBD or Nucleocapsid recombinant antigen is adsorbed onto the 96-well microplate. Following incubation, the microplate is washed to remove unbound antigen and blocked to prevent non-specific binding. Standard, controls and sample dilutions are incubated in the coated microplate in which anti-SARS-CoV-2 Spike, RBD or Nucleocapsid IgG specific antibodies (primary antibodies) bind to the coated antigen.

Following incubation, the microplate is washed to remove unbound primary antibodies. Primary antibodies are detected with the addition of the anti-human IgG antibody (secondary antibody) conjugated to peroxidase. After incubation, the microplate is washed to remove unbound secondary antibodies.

The peroxidase substrate solution, tetramethylbenzidine (TMB), is added to the microplate and a colored product is developed which is proportional to the amount of SARS-CoV-2 Spike, RBD or Nucleocapsid IgG antibodies present in the serum sample. 2N H_2SO_4 is then added to stop the colorimetric reaction.

The absorbance of each well is measured using a microplate spectrophotometer reader at a specific wavelength (450/620 nm). A standard on each tested plate is used to calculate the amount of antibodies against SARS-CoV-2 Spike, RBD or Nucleocapsid in the sample according to the unit assigned by the standard (ELU/mL).