Serological ELISA SARS-Cov-2, Detection of IgG antibodies against RBD

0.5 µg/ml *SARS-CoV-2 Spike S1 Protein (RBD)* (trenzyme GmbH, Germany) was diluted in 1-fold *Coating Buffer pH 7.4* (CANDOR Bioscience GmbH, Germany, # 120, 10-fold-solution) and 100 µl of the solution was added to each well of 96-well ELISA plates *Nunc MaxiSorp* and incubated overnight at 2-8°C. The wells were emptied without washing and blocked with 200 µl *PlateBlock™* (CANDOR Bioscience GmbH, Germany, # 112) per well by incubating for 1 hour at room temperature. The wells were emptied without washing and stabilized with 200 µl per well *Liquid Plate Sealer®* (CANDOR Bioscience GmbH, Germany, # 160) for 1 hour at room temperature. *[The stabilization of the RBD is necessary if the coated ELISA-plates have to be stored for a longer period of time. RBD contains structural epitopes that denature within a few days after immobilization if not stabilized with Liquid Plate Sealer®. If the plates are used directly after immobilization, stabilization is not necessary].*
Coated plates were emptied without washing and air-dried and sealed in aluminium pouches and stored at 2-8°C until use. The plates are stable for many months because of the stabilizing effect of *Liquid Plate Sealer®*.
Human plasma or serum samples were diluted 1:100 with *LowCross-Buffer®* (CANDOR Bioscience GmbH, Germany, # 100). Each serum sample should be tested in duplicates. 100 µl per well of appropriately diluted sample was added to each well of the coated plate and incubated for 2 hours at room temperature with constant shaking on a shaker. The wells were emptied and washed three times with 300 µl for each well with 1-fold *Washing Buffer TRIS™* (CANDOR Bioscience GmbH, Germany, # 145, 10-fold-solution).
100 µl of appropriately diluted (0.25 µg/ml) *goat anti-human IgG peroxidase conjugate* (Jackson ImmunoResearch, USA, #109-035-098) in *HRP-Protector™* (CANDOR Bioscience GmbH, Germany, # 222) was added to the respective wells and incubated for another 1 hour at room temperature with constant shaking. The wells were emptied and washed three times with 300 µl for each well with 1-fold *Washing Buffer TRIS* (CANDOR Bioscience GmbH, Germany, # 145) before addition of 100 µl per well TMB substrate solution *SeramunBlau® slow 50* (Seramun Diagnostica GmbH, Germany, # S-150-TMB). The chromogenic development was stopped using 50 µl per well 1 M H_2SO_4 after 5-10 minutes of light-protected incubation. Optical density (OD) was measured at 450 nm wavelength in a microplate spectrophotometer.

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Obviously this protocol can be a basis for similar assay protocols.
Aspects which can be changed without necessarily making the assay results less reliable:
Coating Stabilizer: *Liquid Plate Sealer*® (CANDOR Bioscience GmbH, Germany, # 160) can be exchanged by *Liquid Plate Sealer*® *animal-free* (CANDOR Bioscience GmbH, Germany, # 163). Both have been tested with same results in the assay and stability testing is ongoing with same results as well. So both solutions can be exchanged by each other.
Secondary antibody: The secondary antibody can be exchanged by other secondaries. But this aspect is critical in terms of choice of the secondary. A secondary for a SARS-CoV-2 IgG-ELISA (the same applies for any other serological IgG ELISA) should not only be specific and of high affinity for human IgG. Furthermore it should be pre-adsorbed against other human antibodies such as IgA, IgM and so on. The reason is that otherwise an IgG-ELISA, intended to detect immunity (to speak in general) would also show IgA-antibodies, which are not a sign of immunity, but a sign of an infection. The same applies vice versa to IgA- or IgM- detecting serological assays. Choice of pre-adsorbed secondaries is absolutely crucial for clinical correctness of results. There are only very few antibody producers with good experience in pre-adsorption. We found in in-house testing at CANDOR several commercially available secondaries, which were named as pre-adsorbed against other antibodies (and maybe they were pre-adsorbed by the producers) but detected additionally the wrong types of human antibodies. This has to be tested by specific assays for this kind of non-specificity to the target. It is not a question of cross-reactivity, but a question of having a mixture of antibodies specific to IgG, specific to IgA, specific to IgM and so on. Due to this such secondaries with bad pre-adsorption show very high affinity binding to the other types of human antibodies. If one likes to save for such additional testing, we recommend using the same secondary as mentioned in the protocol.
Conjugate diluent: *HRP-Protector*™ (CANDOR Bioscience GmbH, Germany, # 222) is used in this protocol instead of *LowCross*® *HRP-Stab* (CANDOR Bioscience GmbH, Germany, # 222). Both options are possible. We tested it and found not one sample showing different results in both. In the protocol *HRP-Protector*™ is mentioned due to a slightly lower cost for this. Using *LowCross*® *HRP-Stab* enables to avoid a specific kind of interference, which can only occur in the last step of the sequential assay protocol. This is by far not the major cause for false positives in serological assays and very seldomly. In our trials we didn't find samples showing this specific kind of interference and so we mention *HRP-Protector*™ for this protocol only for cost reasons. The protocol was developed for commercial kit producers worldwide, which are - due to the high volumes of the Covid-19 IgG ELISA, which will be

produced during the next months - under high cost pressure and this was the basis for deciding to mention *HRP-Protector*™ instead of *LowCross*® *HRP-Stab* for this protocol. For applications with lower volumes, such as in research settings or in clinical testing for drugs or for vaccines we highly recommend using *LowCross*® *HRP-Stab* for even better safety of the protocol.
Substrate: Even other substrates may work well with this ELISA protocol. Choosing an appropriate substrate is a task for itself, but exchange will be possible without risk of worse reliability, if one gets a substrate with similar characteristics as described for this substrate. The substrate is not the most critical component for a reliable immunoassay, but can have bad effects, if it is not well-chosen.
ELISA plate: Potentially other ELISA plates may perform well enough. Our testing showed supremacy of the chosen plate for this specific antigen.
Other changes: We do not recommend any other change on the protocol.
Exchange of surface blocker and assay diluent would be the worst option, as this both leads definitely to high numbers of false positives.

If the protocol should be adapted to IgA ELISA, changes will be necessary not only in terms of the appropriate antigen, but additionally in terms of the appropriate diluents and stabilizers and secondaries, of course. The main reason for such changes is that the one kind of assays needs premium specificity whilst the other kind needs premium sensitivity. Currently we are preparing a similar protocol for high-quality anti-IgA ELISAs for Covid-19 for optimum detection of ongoing infection in people. If you are interested in this protocol, please ask your distributor to be informed, when this protocol will be available.

Kind regards

Your team of CANDOR Bioscience – The ELISA experts