Human ACE2 ELISA kit

Cat#: orb864287 (ELISA Manual)

Test principle

The ELISA Kit produced by Biorbyt adopts the "sandwich method": the capture antibody is coated on the enzyme plate, and the target protein in the sample and standard sample is captured. The biotinylated detection antibody is combined with the target protein, and the SABC complex is combined with the biotinylated detection antibody to form an immune complex. After the TMB chromogenic solution is added, if there is a target protein in the reaction pore, it will be blue, Add the termination solution to turn yellow, and the free components are washed away during the detection process. The OD value is measured at 450 nm with the microplate reader. The concentration of target protein is proportional to the OD value. The concentration of target protein in the sample is calculated by drawing the standard curve.

Kit components & Storage （An unopened kit can be stored at 4°C）

Item	Specifications（48 T）	Specifications（96 T）
Microplate (Dismountable)	8 wells×6 strips	8 wells×12 strips
Standard	1 vials	1 vials
Standard & Sample Diluent Buffer	10ml	15ml
Biotinylated Antibody(100×)	1 vials	1 vials
Biotinylated Antibody Diluent Buffer	6ml	12ml
Avidin-Biotin-Peroxidase Complex（SABC）	6ml	12ml
TMBSolution（A）	3ml	6ml
TMBSolution（B）	3ml	6ml
Stop Solution	6ml	12ml
Wash Buffer (20×)	30ml	30ml ×2
Sealing paper	2	4
Instruction manual	1	1

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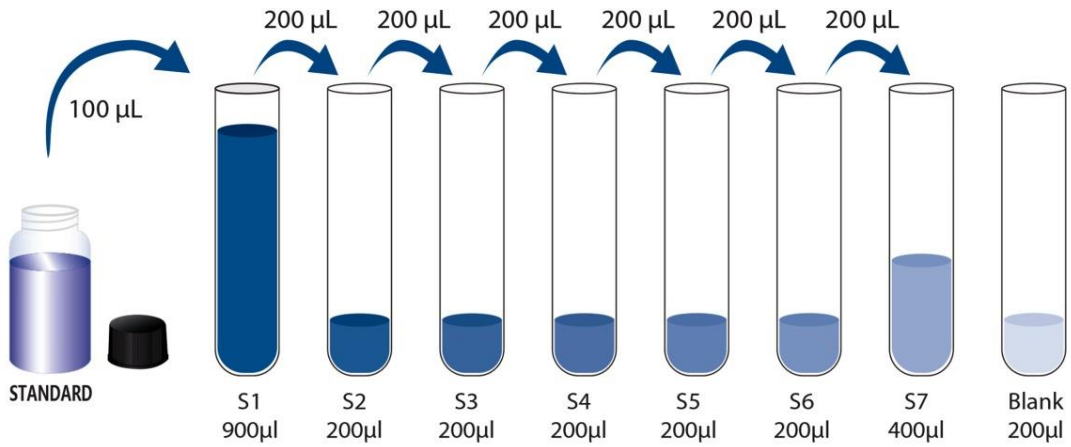
Application: this kit is used for qualitative or quantitative analysis in vitro, only for scientific research, not for clinical diagnosis, and for serum, plasma, tissue homogenate， Cell culture supernatant and other biological fluid.

Specimen collection and reagent preparation:

1. Disposable pyrogen-free and endotoxin-free test tubes (EDTA, citrate and heparin can be anticoagulated) should be used for collecting serum and plasma samples. Hemolysis and hyperlipemia samples should be avoided for serum and plasma, and suspended solids in the samples should be removed by centrifugation to make the samples clear and transparent. Samples to be tested should be detected as soon as possible and stored at 2-8℃ for 48 hours; It should be frozen (-20℃ or -80℃) for a longer time to avoid repeated freezing and thawing.
2. **Wash Buffer:** dilute with distilled water at 1:20 (for example, 1ml concentrated washing liquid is added with 19ml distilled water).
3. **Standards:**

Label 8 EP tubes withS1,S2,S3,S4,S5,S6，S7,blankrespectively.Add 900ul of standard / sample diluent into the first tube S1, 200ul of standard / sample diluent into the second to eighth tubes, add 100ul of (2500pg/ml)standard solution into the first tube S1, put it on the vortex mixer, mix well, suck out 200ul with the sampler and move it to the second tube, so as to double dilute, suck out 200ul from the seventh tube and discard it, and the eighth tube is the blank control（It is suggested to use the concentration in the standard curve : 250、125、62.5、31.25、15.625、7.8125、3.90625、0pg/ml）



Note: It is best to use Standard Solutions within 2 hours.

4. **Preparation of biotinylated antibody working solution**: 20 minutes before use, dilute 100× biotinylated antibody into 1× working solution with biotinylated antibody diluent, prepare according to the required dosage, use it on the same day, and discard the rest.

5. **Configuration of TMB color developing solution:** 10 minutes before use, mix TMB solution A and B 1:1, and keep away from light for standby.
6. If the concentration of the target protein in the sample you tested is higher than the maximum value of the standard, it is recommended to re-test. Please dilute it appropriately according to the actual situation (pre-experiment is recommended to determine the dilution multiple).

Assay Procedure

1. **Sample addition:** Blank wells to 50 ul standard/sample diluent Buffer, 50 ul of standard or sample to be tested were added to the other wells, the reaction plate was mixed well and then placed at 37 ℃ for 40 minutes.
2. **Wash:** Remove the cover and discard the plate content, Use 1 × Wash the reaction plate with washing solution for 4-6 times, and add 1 × Lotion 350 μ l. Shake / soak for 1-2 minutes each time, and print dry on the filter paper..
3. **Biotinylated Antibody:** Add 100ul Biotinylated Antibody working solution into above wells (blank wells addition standards/sample diluent buffer). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 30 minutes.
4. **Wash:** The washing steps are the same as above.
5. **Avidin-Biotin-Peroxidase Complex（SABC）:** Add 100μl of SABC Working Solution into each well, cover the plate and incubate at 37°C for 20 minutes.
6. **Wash:** The washing steps are the same as above.
7. **TMB Solution:** Add 100μl TMB Solution into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
8. **Stop:** Add 100ul stop solution into each well, mix well, and measure the absorbance at 450nm within 30 minutes.**OD**.

   Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Kit performance:

1. sensitivity: the detectable concentration is less than 0.78ng/ml.

2. Specificity: Recombinant or natural Human ACE2 can be detected at the same time without cross reaction with other cytokines in Humans.

3. Repeatability: the coefficient of variation within and between plates is less than 10%.

Matters need attention

1. Double-hole testing is recommended when testing standard products and samples in the test, and standard curves should be made every time.

2. Washing process is critical. Inadequate washing will lead to error in accuracy and error in OD value. It is a normal phenomenon that concentrated washing liquid taken out from refrigerator may have crystallization. Prepare washing liquid after the crystallization is completely dissolved in water bath at 37℃.

3. When testing, all reagents should be restored to room temperature. After opening the slats, the remaining slats should be sealed and put back in the bag for use within one month.

4. The reagent kit uses hypersensitive TMB solution, which will precipitate when the color is too deep, which is a normal phenomenon. It can be mixed evenly without affecting the interpretation of the results.

5. The manual is subject to the paper version in the kit. The kit is only used for scientific research, not for clinical diagnosis!