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11/20

SARS-CoV-2 RBD ELISA Kit

(Catalog # E4877-100, 96 assays, Store at 4ºC)

I. Introduction:

COVID-19 disease is a respiratory illness caused by a novel coronavirus called systemic acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV-2 belongs to the family of β-coronavirus. Its genome is about 30 kb long and shares about 80% of genome identity with SARS-CoV. The 3' proximal terminal consists of 4 structural proteins: Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N). The S protein is a homo-trimeric, class I fusion, transmembrane glycoprotein. It promotes attachment, fusion, and entry of the virus into the host cells. The protein consists of two functional subunits, S1 and S2. The S1 subunit contains the N-terminal domain (NTD) and the C-terminal domain (CTD). The C-terminal domain consists of the receptor-binding domain (RBD) that binds and fuses to the angiotensin-converting enzyme 2 (ACE2) receptor located on the host cells and thus enables entry of the virus into the host. Due to its critical role in viral entry into the host cell, the RBD protein could serve as an efficient target antigen for the development of vaccines. BioVision's SARS-CoV-2 RBD ELISA Kit is designed to quantitatively measure the amount of RBD protein in bronchoalveolar lavage fluid and nasopharyngeal swab samples. The assay is based on the Sandwich ELISA principle. Test samples, Standards, and Biotin-conjugated antibody are added to the wells pre-coated with the RBD antibody and then washed with Wash Buffer. The HRP-Streptavidin is added and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of TMB-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the concentration of RBD in the sample or standard.

II. Features and Benefits:

- Detection range: 12.5 – 800 pg/ml
- Sensitivity: < 10 pg/ml
- Assay Precision: Intra-Assay CV < 12% and Inter-Assay CV < 15%
- This Sandwich ELISA is highly sensitive and highly specific for the detection of RBD protein in bronchoalveolar lavage fluid and nasopharyngeal samples

III. Sample Type:

Bronchoalveolar lavage fluid and nasopharyngeal samples

IV. Kit Contents:

Components	E4877-100	Part Number
Microtiter ELISA plate	8 x 12 Strips	E4877-100-1
SARS-CoV-2 RBD Standard (1600 pg)	1 bottle	E4877-100-2
Standard/Sample Dilution Buffer	25 ml	E4877-100-3
RBD Biotin Conjugated Antibody	120 µl	E4877-100-4
Reagent Dilution Buffer	25 ml	E4877-100-5
HRP-Streptavidin Conjugate (SABC)	120 µl	E4877-100-6
TMB Substrate Solution	12 ml	E4877-100-7
Stop Solution	6 ml	E4877-100-8
Wash Buffer (20X)	25 ml	E4877-100-9
Plate Sealer	4	E4877-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable pipette tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

Store the unopened kit at 4°C. If kit is opened, it must be used within 1 month. Do not use the kit beyond the expiration date

VII. Reagent and Sample Preparation:

Note: Prepare fresh reagents before the start of the experiment

Before using the kit, spin tubes and bring down all components to the bottom of tubes

Bring all reagents to room temperature prior to use

1. **Biotin-labeled Antibody working solution:** Prepare a working stock solution of SARS-CoV-2 RBD Biotin-conjugate antibody by diluting the antibody in Reagent Dilution Buffer at 1:100. For example, dilute 100 µl of Biotin-conjugate antibody in 9,900 µl Reagent Dilution Buffer to prepare a 10 ml working stock of Biotin conjugated antibody solution.
2. **HRP-Streptavidin Conjugate (SABC):** Prepare a working stock solution of HRP-Streptavidin conjugate by diluting the conjugate in Reagent Dilution Buffer at 1:100. For example, dilute 100 µl of HRP-Streptavidin conjugate in 9,900 µl Reagent Dilution Buffer to prepare a 10 ml working stock of HRP-Streptavidin conjugate solution.
3. **Wash Buffer:** Dilute 20X Wash Buffer to 1X by adding 20 ml of 20X Wash Buffer to 380 ml deionized/distilled water to prepare 400 ml of 1X Wash Buffer solution. If crystals are present in the 20X Wash Buffer, warm it at room temperature. Mix it gently to dissolve the crystals.
4. **Standard Preparation:**
   - Add 2 ml Standard/Sample Dilution Buffer to reconstitute the SARS-CoV-2 RBD standard. The concentration of the standard

after reconstitution will be 800 pg/ml. This can be labeled as a High Standard. Prior to making dilutions, mix the standard solution well and allow it to sit for 10 minutes.

- Label 7 tubes as 400 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 12.5 pg/ml and 0 pg/ml respectively. Add 400 µl of the Standard/Sample Diluent Buffer into Tubes 1-7. Pipette out 400 µl from 800 pg/ml Stock Solution and add to Tube 1. Mix thoroughly.
- Transfer 400 µl from Tube 1 to Tube 2 and mix well. Transfer 400 µl from Tube 2 to Tube 3 and mix and so on. Tube 7 will only have 400 µl of the Standard/Sample Dilution Buffer which will serve as Zero Standard (0 pg/ml).

VII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve should be run for each assay.

1. Prepare all reagents, samples and standards as instructed in **section VII**.
2. Any unused microplate strips must be removed from the plate frame and returned to the foil pouch that contains desiccant pack and must be resealed.
3. Add 100 µl of each **Standards** (0 – 800 pg/ml) or **Samples** into appropriate wells. Cover the wells with the adhesive strip and incubate for 1 hour at 37ºC.
4. Remove the adhesive strip and aspirate the plate contents. Wash the plate 3 times with 300 µl **1X Wash Buffer**. Wash by filling each well with **1X Wash Buffer** using a multi-channel pipette or auto-washer and then remove all residual **Wash Buffer** from the wells by aspiration. After the last wash, remove any remaining **Wash Buffer** by aspirating or decanting. Tap the plate on absorbent filter papers.
5. Add 100 µl of **RBD Biotin-conjugated antibody** work solution into the above wells. Seal the plate and incubate at 37ºC for 30 minutes.
6. Discard the solution and wash 3 times with **1X Wash Buffer**.
7. Add 100 µl of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 minutes.
8. Discard the solution and wash 3 times with **1X Wash Buffer** as step 6.
9. Add 100 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark for 10 minutes. Protect the plate from light
10. Add 50 µl of **Stop Solution** to each well. The color should change from blue to yellow. If the color changes to green or appears non-uniform, gently tap the plate to ensure thorough mixing. Read result at 450 nm within 30 minutes. If wavelength correction is available, then set to 540 or 570 nm. If wavelength correction is not available, then subtract 540 nm/ 570 nm from 450 nm. The subtraction will correct for any optical interference in the plate. If wavelength correction is not performed, the readings at 450 nm may be higher and less accurate.

VIII. Calculation:

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero 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. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

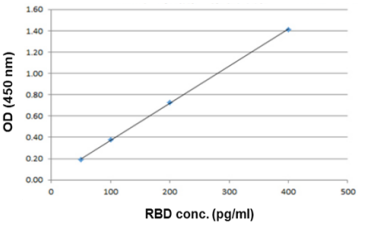

Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

IX. Related Products:

- Coronavirus Rapid RT-qPCR Detection Kit (K1461)
- SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit
- Coronavirus (SARS-CoV-2) PCR Detection Kit (K1460)
- SARS-CoV-2 IgG ELISA Kit
- SARS-CoV-2 IgM ELISA Kit