Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

SARS-CoV-2 RBD ELISA Kit

rev 03/21

(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 Detection A solution are added to the wells pre-coated with the RBD antibody and then washed with Wash Buffer. The Detection B solution is added and any unattached conjugates are washed off using Wash Buffer. The 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	Storage
Microtiter ELISA plate	8 x 12 Strips	E4877-100-1	-20°C
SARS-CoV-2 RBD Standard (40 ng)	1 bottle	E4877-100-2	-20°C
Standard/Sample Dilution Buffer	25 ml	E4877-100-3	4°C
Detection A	1 vial	E4877-100-4	-20°C
Reagent Dilution Buffer	25 ml	E4877-100-5	4°C
Detection B	1 vial	E4877-100-6	-20°C
TMB Substrate Solution	12 ml	E4877-100-7	4°C
Stop Solution	6 ml	E4877-100-8	4°C
Wash Buffer (20X)	25 ml	E4877-100-9	4°C
Plate Sealer	4	E4877-100-10	RT

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:

For the unopened kit, store the **Microplate, Standard, Detection A, and Detection B** at -20°C, the rest of the reagents must be stored at 4°C. When the kit is stored at the recommended temperature for 6 months, the intensity of the signal decreases by less than 10%. 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. **Detection A working solution:** Shake and mix the vial before use. Centrifuge the vial to bring the liquid to the bottom of the tube. Dilute **Detection A** in **Reagent Dilution Buffer** at a ratio of 1:2000.
2. **Detection B working solution:** Shake and mix the vial before use. Centrifuge the vial to bring the liquid to the bottom of the tube. Dilute **Detection B** in **Reagent Dilution Buffer** at a ratio of 1:2000.
3. **Wash Buffer (1X):** Prepare **Wash Buffer (1X)** by adding 25 ml of **Wash Buffer (20X)** to 475 ml deionized/distilled water to prepare 500 ml of **Wash Buffer (1X)**. If crystals are present in the **Wash Buffer (20X)**, warm it at room temperature. Mix it gently to dissolve the crystals.
4. **Standard Preparation:**
   - Add 0.5 ml **Standard/Sample Dilution Buffer** to produce a stock solution of **80 ng/ml**. Prior to making dilutions, gently mix

the standard solution well and allow it to sit for 15 minutes.

- Dilute the **80 ng/ml** stock solution 100 times with **Standard/Sample Dilution Buffer** to obtain the first standard (**800 pg/ml**). Prepare serial dilutions of subsequent standards to obtain following concentrations: **400 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, and 12.5 pg/ml**. Zero Standard (**0 pg/ml**) will only contain **Standard/Sample Dilution Buffer**.

5. **Sample Preparation:**
   - **Cell culture supernatants:** Centrifuge the sample to settle down the particulates. Use this sample immediately for the assay. If samples cannot be used immediately, then aliquot and store at ≤ -20°C. Avoid freeze/thaw cycles.

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**), **Controls** (if any) 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 **Wash Buffer (1X)**. Wash by filling each well with **Wash Buffer (1X)** using a multi-channel pipette or auto-washer and then remove all residual **Wash Buffer (1X)** from the wells by aspiration. After the last wash, remove any remaining **Wash Buffer (1X)** by aspirating or decanting. Tap the plate on absorbent filter papers.
5. Add 100 µl of **Detection A working solution** into each of the wells. Seal the plate and incubate at 37ºC for 1 hour.
6. Discard the solution and wash 3 times with **Wash Buffer (1X)** as in **step 4**.
7. Add 100 µl of **Detection B working solution** into each well, cover the plate and incubate at 37ºC for 30 minutes.
8. Discard the solution and wash 3 times with **Wash Buffer (1X)** as in **step 4**.
9. Add 100 µl of **TMB substrate** into each well, cover the plate and incubate at room temperature (RT) in dark for 20 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:

Average the duplicate readings for standard, control, and samples. 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.

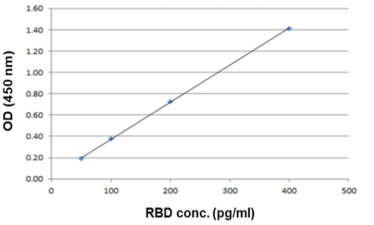

Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

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