

ab289833 – SARS-CoV-2 RBD ELISA Kit

For the quantification of RBD protein in bronchoalveolar lavage fluid and nasopharyngeal swab samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab289833>

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
Microtiter ELISA Plate	8 x 12 wells	-20°C
SARS-CoV-2 RBD Standard	1 bottle	-20°C
Sample / Standard dilution buffer	25 mL	4°C
Detection A	1 vial	-20°C
Reagent dilution buffer	25 mL	4°C
Detection B	1 vial	-20°C
TMB substrate (Avoid light)	12 mL	4°C
Stop Solution	6 mL	4°C
Wash buffer (25X)	25 mL	4°C
Plate sealers	4 units	Room Temperature

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.
- Bring all reagents to room temperature prior to use.

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.

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.

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.

Standard Preparation:

1. 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.
2. 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, 200, 100, 50, 25, and 12.5 pg/mL. Zero Standard (0 pg/mL) will contain only Standard/Sample Dilution Buffer.

Sample Preparation:

Cell culture supernatant: 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.

Assay Protocol

- 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 must be run with each assay.
1. Prepare all reagents, samples and standards as instructed in Reagent Preparation section.
 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 37°C.
 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 37°C 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 37°C 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.

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.

Technical Support

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