

ab284402 – Covid-19 S-protein (S1RBD) ELISA kit

For the quantitative measurement of Covid-19 S-protein (S1RBD) in serum, cell culture supernatant samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab284402

Storage and Stability: The entire ELISA kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. Observe the storage conditions for individual prepared components in the Reagent Preparation.

Materials Supplied

Item	Quantity	Storage Condition
Microplate	96 wells	-20°C
Wash Buffer Concentrate (20X)	25 mL	-20°C
Standard Protein	2 vials	-20°C
Detection Antibody	2 vials	-20°C
HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C
Assay Diluent A	30 mL	-20°C
Assay Diluent B	15 mL	-20°C

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.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

Reagents Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

Assay Diluent B: Dilute 5-fold with distilled or deionized water before use. Once diluted can be stored for 1 month at 4°C.

Sample Dilution: Assay Diluent A should be used for dilution of serum samples. 1X Assay Diluent B should be used for dilution of culture supernatants. The suggested dilution for normal serum/plasma is 4-fold.

▲ Note: Levels of COVID-19 S-Protein (S1RBD) may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Wash Buffer Concentrate (20X): If it contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer. Once diluted, can be stored for 1 month at 4°C.

Detection Antibody: Biotinylated anti-Virus COVID-19 S-Protein. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B to make 1X Prepared Biotinylated Antibody. This is used in step 4 of the Assay Procedure.

HRP-Streptavidin Concentrate: Briefly spin and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent B, mix well. Once diluted, do not store and reuse.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 50 µL of HRP-Streptavidin concentrate into tube with 10 mL 1X Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin solution.

Standard Preparation

- Always prepare a fresh set of standards for every use.
 - Discard working standard dilutions after use as they do not store well.
 - The following section describes the preparation of a standard curve for duplicate measurements (recommended).
1. Briefly spin a vial of Standard Protein. Add 400 µL Assay Diluent A (for serum samples) or 1X Assay Diluent B (for cell culture supernates) into Standard Protein vial to prepare a 25 ng/mL standard.
 2. Dissolve the powder thoroughly by a gentle mix.
 3. Add 30 µL of the standard solution from the vial of Standard Protein, into a tube with 470 µL 1X Assay Diluent A (for serum samples) or 1X Assay Diluent B (for cell culture supernates) to prepare a 1500 pg/mL standard solution.
 4. Pipette 300 µL Assay Diluent A or 1X Assay Diluent B into each tube.
 5. Use the 1500 pg/mL standard solution to produce a dilution series (shown in the table below).
 6. Mix each tube thoroughly before the next transfer.
 7. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL).

Tube #	Volume to dilute	Volume of 1X Assay Diluent	Final Concentration pg/mL
1	30 µL Standard Stock Solution	470 µL	1500
2	200 µL of tube #1	300 µL	600
3	200 µL of tube #2	300 µL	240
4	200 µL of tube #3	300 µL	96
5	200 µL of tube #4	300 µL	38.40
6	200 µL of tube #5	300 µL	15.36
7	200 µL of tube #6	300 µL	6.14
8	---	300 µL	0

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

▲ Note: 1X Assay Diluent should be used for dilution of serum, and cell culture supernatant samples. The suggested dilution for normal serum is at least 4-fold.

1. Label removable 8-well strips as appropriate for your experiment.
2. Add 100 µL of each standard and sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.
3. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of 1X Prepared Biotinylated Antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µL of prepared Streptavidin solution to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Calculation

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical data

SENSITIVITY

The minimum detectable dose of Virus COVID-19 S-Protein (S1RBD) was determined to be 4.5 pg/ml. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

SPIKING & RECOVERY

Recovery was determined by spiking various levels of Virus COVID-19 S-Protein (S1RBD) into the sample types listed below. Mean recoveries are as follows:

Sample type	Average % recovery	Range %
Serum	104.6	94-117
Cell Culture Media	105.3	91-113

LINEARITY

Sample type	Serum	Cell Culture Media
1:2 Average % of Expected Range (%)	92.26 90-94	95.43 93-98
1:4 Average % of Expected Range (%)	79.23 77-82	74.92 72-78

REPRODUCIBILITY

Intra-Assay CV%: <10%
Inter-Assay CV%: <12%

SPECIFICITY

This ELISA antibody pair detects COVID-19 S Protein (S1RBD)

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For all technical or commercial enquiries please go to:

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