Version 01-06/20

User's Manual

SARS-CoV-2 Spike glycoprotein ELISA Kit

REF **DEIASL018**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The kit has been verified by high purity SARS-CoV-2 (2019-nCoV) Spike S1 Protein and SARS-CoV-2 (2019-nCoV) Spike Pseudoviruses.

The use of this kit for natural samples need be validated by the end user due to the complexity of natural targets and unpredictable interference.

General Description

The spike (S) glycoprotein of coronaviruses contains protrusions that will only bind to certain receptors on the host cell. Known receptors bind S1 are ACE2, angiotensin-converting enzyme 2; DPP4, dipeptidyl peptidase-4; APN, aminopeptidase N; CEACAM, carcinoembryonic antigen-related cell adhesion molecule 1; Sia, sialic acid; O-ac Sia, O-acetylated sialic acid. The spike is essential for both host specificity and viral infectivity. The term 'peplomer' is typically used to refer to a grouping of heterologous proteins on the virus surface that function together. The spike (S) glycoprotein of coronaviruses is known to be essential in the binding of the virus to the host cell at the advent of the infection process. It's been reported that 2019-nCoV can infect the human respiratory epithelial cells through interaction with the human ACE2 receptor. The spike protein is a large type I transmembrane protein containing two subunits, S1 and S2. S1 mainly contains a receptor binding domain (RBD), which is responsible for recognizing the cell surface receptor. S2 contains basic elements needed for the membrane fusion.The S protein plays key parts in the induction of neutralizing-antibody and T-cell responses, as well as protective immunity. The main functions for the Spike protein are summarized as: Mediate receptor binding and membrane fusion; Defines the range of the hosts and specificity of the virus; Main component to bind with the neutralizing antibody; Key target for vaccine design; Can be transmitted between different hosts through gene recombination or mutation of the receptor binding domain (RBD), leading to a higher mortality rate.

Principles of Testing

The principle of this ELISA kit is based on the solid phase sandwich enzyme immunoassay technique. A monoclonal antibody specific for SARS-CoV-2 (2019-nCoV) Spike has been pre-coated onto well plate strips. Standards and samples are added to the wells and SARS-CoV-2 (2019-nCoV) Spike present in the sample is bound by the immobilized antibody. After incubation the wells are washed and a horseradish peroxidase conjugated anti-SARS-CoV-2 (2019-nCoV) Spike antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody a TMB substrate solution is loaded and color develops in proportion to the amount of SARS-CoV-2 (2019-nCoV) Spike bound. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm (See schematics below).

Reagents And Materials Provided

1. SARS-CoV-2 (2019-nCoV) Spike Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with chimeric mAb antibody against SARS-CoV-2 (2019-nCoV) Spike.
2. SARS-CoV-2 (2019-nCoV) Spike Detection Antibody - 0.2 mg/mL of chimeric mAb antibody against SARS-

CoV-2 (2019-nCoV) Spike conjugated to horseradish peroxidase (HRP) with preservatives.

3. SARS-CoV-2 (2019-nCoV) Spike Standard - Recombinant SARS-CoV-2 (2019-nCoV) Spike in a buffer with preservatives, lyophilized. The amount of standard is lot specific and indicated on the label of standard vial.
4. Wash Buffer Concentrate - 25 mL of a 20-fold concentrated solution of buffered surfactant with preservatives.
5. Dilution Buffer Concentrate - 8 mL of a 20-fold concentrated dilution buffer with preservatives.
6. 1×Standard Dilution Buffer - 2 mL
7. Color Reagent A - 13 mL of stabilized hydrogen peroxide
8. Color Reagent B - 13 mL of stabilized chromogen (tetramethylbenzidine)
9. Stop Solution - 8 mL of 2 N sulfuric acid

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Pipettes and pipette tips
3. Deionized or distilled water
4. Multi -channel pipette, squirt bottle, manifold dispenser, or automated microplate washer
5. 500 mL graduated cylinder
6. Tubes for standard dilution
7. Well plate cover or seals

Storage

Store at 2 - 8°C and the kit is stable for 6 months upon receipt.

Specimen Collection And Preparation

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or lower temperature. Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and storesamples at -20° C. Avoid repeated freeze-thaw cycles.

Note: The sample should be diluted to within the working range of the assay in 1× dilution buffer. The exact dilution must be determined based on the concentration of specific target in individual samples.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1×wash buffer by adding 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 400 mL of Wash Buffer.

Dilution Buffer - Prepare 1×dilution buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

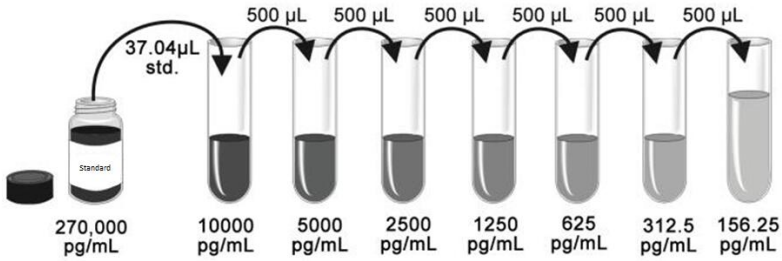
Detection Antibody - Centrifuge at 10,000 x g for 20 seconds. Dilute to work concentration of 0.12 μg/mL in Dilution Buffer before use.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well. Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.

SARS-CoV-2 (2019-nCoV) Spike Standard - Reconstitute the SARS-CoV-2 (2019-nCoV) Spike Standard with 1 mL of 1×Standard Dilution Buffer to make stock solution. Shake the vial gently until the lyophilized powder totally dissolved (Do not turn the vial upside down). Mix the standard to ensure complete reconstitution prior to making dilutions.

Prepare serially diluted standards as described in the following step:

Pipette 962.96 μL of Dilution Buffer into the 10000 pg/mL tube. Pipette 500 μL of Dilution Buffer into the remaining tubes. Use the stock solution to produce a dilution series as the following figure. Mix each tube thoroughly before the next transfer. The 10000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days. The following graph is only for demonstration purposes. The concentration of stock solution is lot specific and need be calculated with the actual amount of standard labeled on the standard vial.



Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Wash each well three times with Wash Buffer (300 μL/well) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μL of each serially diluted protein standard or test sample per well including a zero standard. Ensure reagent addition is uninterrupted and completed within 15 minutes. Cover/seal the plate and

incubate for 2 hours at room temperature.

5. Repeat the aspiration/wash as in Step 3.
6. Add 100 µL of Detection Antibody in working concentration to each well. Cover/seal the plate and incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in Step 3.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Protect from light.
9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 20 minutes, using a microplate reader set to 450 nm.

Calculation

If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

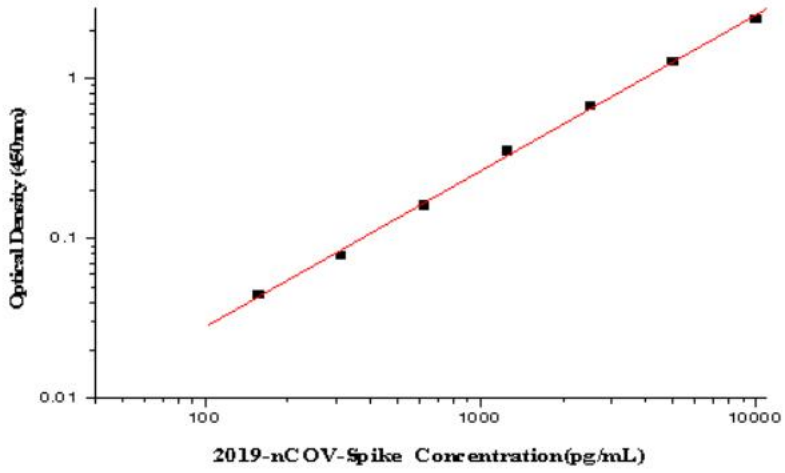
Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.) .

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. Most graphing software can help make the curve and a four parameter logistic (4-PL) usually provide the best fit, though other equations (e.g. linear, log/log) can also be tried to see which provides the most accurate. Extrapolate the target protein concentrations for unknown samples from the standard curve plotted.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
156.25	0.045
312.5	0.078
625	0.162
1250	0.354
2500	0.672
5000	1.290
10000	2.357



Reference Values

The Pseudoviruses (10^{10} copies/mL) expressing SARS-CoV-2 (2019-nCoV) Spike protein on the surface were used as samples.

		EDTA Plasma	Cell culture supernates
1:2	recovery of detected	100%	100%
1:4	recovery of detected	104%	105%
1:8	recovery of detected	100%	109%
1:16	recovery of detected	108%	112%

Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	1663	3078	5852	1615	3121	5652
SD	36.04	59.86	183.92	71.74	50.10	234.80
CV (%)	2.2%	1.9%	3.1%	4.4%	1.6%	4.2%

Sensitivity

The minimum detectable dose (MDD) of SARS-CoV-2 (2019-nCoV) Spike is typically less than 5.55 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes the following factors:

SARS-CoV-2 (2019-nCoV) Spike S1-His Recombinant Protein

SARS-CoV-2 (2019-nCoV) Spike S1+S2 ECD-His Recombinant Protein

SARS-CoV-2 Spike S1-His Recombinant Protein

SARS-CoV-2 Spike S1+S2 ECD-His Recombinant Protein

The factors listed below were prepared at 50 ng/mL in dilution buffer and assayed for cross-reactivity. No cross-reactivity was observed.

MERS-CoV Spike/S1 Protein (S1 Subunit, aa 1-725, His Tag)

Human coronavirus HKU1 (isolate N5) (HCoV-HKU1) Spike Protein (S1 Subunit, His Tag)

Human coronavirus HKU1 (isolate N1) (HCoV-HKU1) Spike/S1 Protein (S1 Subunit, His Tag)

Human coronavirus (HCoV-229E) Spike/S1 Protein (S1 Subunit, His Tag)

Human coronavirus (HCoV-NL63) Spike/S1 Protein (S1 Subunit, His Tag)

Human coronavirus (HCoV-OC43) Spike Protein (S1 Subunit, His Tag)

Influenza A H1N1 (A/California/07/2009) Hemagglutinin / HA Protein (His Tag)

Influenza A H1N1 (A/Michigan/45/2015) Hemagglutinin / HA Protein (His Tag)

Influenza A H3N2 (A/Switzerland/9715293/2013) Hemagglutinin / HA Protein (His Tag)

Influenza A H3N2 (A/Texas/50/2012) Hemagglutinin / HA Protein (His Tag)

Influenza A H5N1 (A/Hong Kong/483/1997) Hemagglutinin / HA Protein (His Tag)

Influenza A H7N9 (A/Anhui/1/2013) Hemagglutinin / HA Protein (His Tag)

Influenza B (B/PHUKET/3073/2013) Hemagglutinin / HA Protein (His Tag)

Influenza B (B/Brisbane/60/2008) Hemagglutinin / HA Protein (His Tag)

MERS-CoV Spike Protein (S1+S2 ECD, aa 1-1297, His Tag)

Human coronavirus (HCoV-NL63) Spike Protein (S1+S2 ECD, His Tag)

Human coronavirus (HCoV-229E) Spike Protein (S1+S2 ECD, His Tag)

Human coronavirus HKU1 (isolate N5) (HCoV-HKU1) Spike Protein (S1+S2 ECD, His Tag)

Human coronavirus (HCoV-OC43) Spike Protein (S1+S2 ECD, His Tag)

Preparations of the factors listed below at 50 ng/mL in a mid-range SARS-CoV-2 (2019-nCoV) Spike S1-His Recombinant Protein (HPLC-verified) control were assayed for interference. No significant interference was observed.

MERS-CoV Spike/S1 Protein (S1 Subunit, aa 1-725, His Tag)

Human coronavirus HKU1 (isolate N5) (HCoV-HKU1) Spike Protein (S1 Subunit, His Tag)

Human coronavirus HKU1 (isolate N1) (HCoV-HKU1) Spike/S1 Protein (S1 Subunit, His Tag)

Human coronavirus (HCoV-229E) Spike/S1 Protein (S1 Subunit, His Tag)

Human coronavirus (HCoV-NL63) Spike/S1 Protein (S1 Subunit, His Tag)

Human coronavirus (HCoV-OC43) Spike Protein (S1 Subunit, His Tag)

Influenza A H1N1 (A/California/07/2009) Hemagglutinin / HA Protein (His Tag)

Influenza A H1N1 (A/Michigan/45/2015) Hemagglutinin / HA Protein (His Tag)

Influenza A H3N2 (A/Switzerland/9715293/2013) Hemagglutinin / HA Protein (His Tag)

Influenza A H3N2 (A/Texas/50/2012) Hemagglutinin / HA Protein (His Tag)

Influenza A H5N1 (A/Hong Kong/483/1997) Hemagglutinin / HA Protein (His Tag)

Influenza A H7N9 (A/Anhui/1/2013) Hemagglutinin / HA Protein (His Tag)

Influenza B (B/PHUKET/3073/2013) Hemagglutinin / HA Protein (His Tag)

Influenza B (B/Brisbane/60/2008) Hemagglutinin / HA Protein (His Tag)

MERS-CoV Spike Protein (S1+S2 ECD, aa 1-1297, His Tag)

Human coronavirus (HCoV-NL63) Spike Protein (S1+S2 ECD, His Tag)

Human coronavirus (HCoV-229E) Spike Protein (S1+S2 ECD, His Tag)

Human coronavirus HKU1 (isolate N5) (HCoV-HKU1) Spike Protein (S1+S2 ECD, His Tag)

Human coronavirus (HCoV-OC43) Spike Protein (S1+S2 ECD, His Tag)

Linearity

		EDTA Plasma	Cell culture supernates
1:2	recovery of detected	100%	100%
1:4	recovery of detected	104%	105%
1:8	recovery of detected	100%	109%
1:16	recovery of detected	108%	112%

Recovery

The recovery of SARS-CoV-2 (2019-nCoV) Spike spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
EDTA Plasma	93	90-99%
Cell culture supernates	104	95-113%

Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the specific targets and samples shown in the manual. The use of

this kit for other purpose should be verified carefully by the end user.

5. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.
6. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
7. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.
8. Bring all reagents and samples to room temperature before use.
9. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
10. A standard curve should be generated for each set of sample assayed. DO NOT USE the standard curves from other plates or other days.
11. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
12. Read the absorbance of each well within 20 minutes after adding the stop solution.