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User's Manual

Human SARS-CoV-2 (Covid-19) Spike Protein S2 Antigen ELISA Kit

REF **DEIAP015-18**

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

Enzyme Immunoassay for the Quantitative Antigen Determination of SARS-CoV-2 (Covid-19) Spike Protein S2 in human serum and plasma and cell culture supernatant.

General Description

The ELISA kit is used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

The spike protein (S-protein) of the SARS-CoV-2 virus is the common target for neutralizing antibodies and vaccines. Spike protein contains two subunits, S1 and S2. S1 contains a receptor binding domain (RBD), which is responsible for recognizing and binding with the cell surface receptor. S2 subunit contains other basic elements needed for the membrane fusion. SARS-CoV-2 (2019-nCoV) can infect the human respiratory epithelial cells through interaction with huamn ACE2. Indeed, the recombinant Spike protein can bind with recombinant ACE2 protein.

Principles of Testing

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human SARS-CoV-2 (Covid-19) spike protein S2 present in the sample are bound by the capture antibodies. After incubation the wells are washed and followed by HRP- conjugated Detection Antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human SARS-CoV-2 (Covid-19) spike proteins in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Reagents And Materials Provided

1. SARS-CoV-2 (Covid-19) spike protein S2 Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Human SARS-CoV-2 spike protein S2 Standards (0.5 ml) - 0, 15.6, 31.25, 125, 250, 500 ng/ml
3. SARS-CoV-2 (Covid-19) Antibody:HRP Conjugate - 12 ml
4. (5X) Assay Diluent - 25 ml
5. (20X) Wash Buffer - 25 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml
8. Instruction Manual
9. Plate Sealer

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Storage

1. Store main kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection And Preparation

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided.

Samples should be diluted 1:5000 (v/v) for optimal recovery, (for example 1 ul sample + 4999 ul sample diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Sample Diluent accordingly.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

Note:

Grossly hemolyzed samples are not suitable for use in this assay.

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.

Note:

The sample should be diluted to within the working range of the assay in 1X Assay Diluent. The exact dilution must be determined based on the concentration of specific target in individual samples.

Reagent Preparation

All reagents should be diluted immediately prior to use:

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.

2. Bring all reagents to Room Temperature before use.
3. To make Assay Diluent (1X); dilute 25 ml of 5X Assay Diluent in 100 ml of DI water.
4. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.

Assay Procedure

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in erroneous results for the presence of Human SARS-CoV-2 (Covid-19) S2 proteins.
3. It is recommended that the Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Pipette 100 ul of Standards and Samples to the respective wells. Seal plate and incubate for 1 hour at Room Temperature (18-25°C).
2. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
3. Add 100 ul of SARS-CoV-2 Antibody:HRP Conjugate to each well.
4. Seal plate and incubate for 1 hour at Room Temperature (18-25°C).
5. Wash plate 4 times with Wash Buffer (1X) as in step 2.
6. Pipette 100 ul of TMB Substrate solution.
7. Incubate in the dark for 30 minutes at Room Temperature.
8. Stop reaction by adding 100 ul of Stop Solution to each well.
9. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Calculation

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding

concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human SARS-CoV-2 (Covid-19) spike protein S2 concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve.

At the point of intersection, draw a vertical line to the X-axis and read the Human SARS-CoV-2 (Covid-19) spike protein S2 Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or a polynomial 2nd order curve is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:

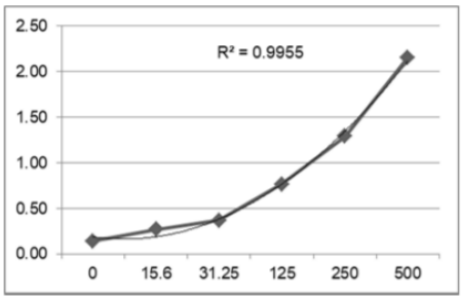
- If the sample absorbance value is below the first standard.

Typical Standard Curve

Typical Data

Standard Concentration (ng/ml)	Abs 1	Abs 2	Mean Absorbance
0	0.155	0.127	0.141
15.6	0.335	0.201	0.268
31.25	0.434	0.300	0.367
125	0.791	0.741	0.766
250	1.324	1.253	1.289
500	2.209	2.100	2.155

Typical Graph



Precision

Intra-Assay

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

Inter-Assay:

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

	Intra-Assay			Inter-Assay		
Sample	1	2	3	1	2	3
N	5	5	5	5	5	5
Mean (ng/ml)	1586	980	457	1590	1293	1725
CV (%)	14.2 %	10.0 %	9.5 %	10.0 %	12.7 %	9.5 %

Detection Range

0 ng/ml - 500 ng/ml

Detection Limit

It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be less than 6.8 ng/ml.

Sensitivity

The capture antibodies used in the kit are specific against 2019-nCoV Coronavirus spike and has 100% crossreactivity with SARS-CoV Spike S2 Protein and some cross-reactivity with SARS-CoV-2 Spike S1+S2 ECD Protein, SARS-CoV Spike S1+S2 ECD Protein, MERS-CoV Spike S1+S2 ECD Protein.

The kit has no cross-reactivity with MERS-CoV Spike Spike S2, SARS-CoV-2 (2019-nCoV) Spike S1, SARSCoV-2 (2019-nCoV) Spike RBD, HCoV-OC43-Spike S1+S2 Protein, HCoV-229E-Spike S1+S2 Protein, HCoVHKU1 (isolate N5)Spike S1+S2 Protein, HCoV-NL63-Spike S1+S2 Protein.

Recovery

SARS-CoV-2 (Covid-19) spike protein S2 was spiked at different levels to measure mean recovery.

Sample	Mean % Recovery	Range
Serum (n=3)	80.4	78-84 %
Cell Culture Supernates (n=3)	114	105-125 %

Precautions

1. This kit is For Research Use Only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept in the original shipping container.
7. Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
8. Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material.

- Always use protective gloves - Never pipette material by mouth.

- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

10. In any case GLP should be applied with all general and individual regulations to the use of this kit.

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