SARS-CoV-2 (Covid-19) Inhibitor Screening ELISA

REF : KBVH300

Ver 1.0

RUO

distributed in the US/Canada by:
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Enzyme Immunoassay for the Qualitative Screening of SARS-CoV-2 Inhibitors in biological samples and human serum

RUO	**For Research Use Only**	**REF**	**Catalog Number**
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Introduction:

The SARS-CoV-2 (Covi-19) Inhibitor Screening ELISA kits are 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 SARS-CoV-2 comprises 4 structural proteins - Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S), which is a transmembrane protein, composed of two subunits S1 and S2. The S1 subunit contains a receptor binding domain (RBD), which binds to the cell surface receptor Angiotensin-Converting Enzyme 2 (ACE2) present at the surface of epithelial cells, causing mainly infection of human respiratory cells.

The SARS-CoV-2 Inhibitor Screening ELISA measures the binding of the RBD of the Spike S protein from SARS-CoV-2 to its human receptor ACE2. Thus, this assay allows to identify and characterize the effect of different inhibitory molecules including antibodies or chemicals on the inhibition of the binding of SARS-CoV-2 virus to any ACE2-expressing cells.

Intended Use:

The SARS-CoV-2 (Covid-19) Inhibitor Screening ELISA is used as an analytical tool for inhibitor screening of SARS-CoV-2 (Covid-19) in biological samples and human serum.

Principle:

The method employs sandwich ELISA technique. Human SARS-CoV-2 S protein RBD is pre-coated onto microwells. Human ACE2 and Inhibitor in the Samples compete and Inhibitor Control are pipetted into microwells. The Inhibitor in the Samples and Control will inhibit the ACE2 from binding to the SARS-CoV-2 s protein RBD to form a complex. After incubation the wells are washed and 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 in inverse proportional to the amount of Inhibitor in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Recombinant SARS-CoV-2 (Covid-19) S spike protein RBD Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Human ACE2 Protein - 12 ml
3. Inhibitor Control (0,5 ml) - 1 vial
4. Anti-His:HRP Conjugate - 12 ml
5. Assay Diluent - 100 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
8. Instruction Manual

Materials to be provided by the End-User:

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

Handling/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.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

Sample Preparation and Storage:

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. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

Preparation before Use:

Allow serum samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

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.*

Biological Samples: Dilute each SARS-CoV-2 inhibitor (chemicals or antibodies) to be tested to the desired final concentration in 100 ul of Assay Diluent.

Note: *Do not exceed 0.1% DMSO if using chemicals as inhibitors*

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 **Wash Buffer (1X)**; dilute **20 ml of 20X Wash Buffer in 380 ml of DI water**.

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. It is recommended that the Standards and Samples be assayed in duplicates.
3. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
4. 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.
5. The plates should be read within 30 minutes after adding the Stop Solution.
6. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Pipette 100 ul of Human ACE2 Protein to all wells.
2. Pipette 100 ul of **Standards** and **Inhibitor Control** to the respective wells.
3. Seal plate and incubate for 1 hour at Room Temperature (18-25ºC).
4. 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.
5. Add 100 ul of **Anti-His:HRP Conjugate** to each well.
6. Seal plate and incubate for 1 hour at Room Temperature (18-25°C).
7. Wash plate 4 times with **Wash Buffer (1X)** as in step 2.
8. Pipette 100 ul of **TMB Substrate solution**.
9. Incubate in the dark for 15 minutes at Room Temperature.
10. Stop reaction by adding 100 ul of **Stop Solution** to each well.
11. Read absorbance at 450 nm within 30 minutes of stopping reaction.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate Sample and Control. Using standard graph paper, plot the % inhibition of each sample on the Y-axis versus the corresponding concentration of the inhibitor concentration on the X-axis. Draw the best fit curve through the standard points.

Software which is able to generate a polynomial regression (2^{nd} order) or a cubic spline curve-fit is best recommended for automated results.

Typical Data

Remdesivir (Inhibitor Control) concentration (ug/ml)	Abs 1	Abs 2	Mean Abs	% Inhibition
0	2.212	2.210	2.211	100.0
0.25	1.878	1.936	1.907	86.3
0.5	1.021	0.968	0.995	45.0
1	0.668	0.645	0.657	29.7
10	0.567	0.562	0.565	25.5

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. When setting up the inhibitor samples as dilution, please ensure the concentrations are within the linear range of the graph.

Safety Precautions:

- **This kit is For Research Use Only**. Follow the working instructions carefully.

- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- 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.
- 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.
- 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.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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