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User's Manual

Human anti-HCoV NL63 IgA ELISA Kit

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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.

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PRODUCT INFORMATION

Intended Use

The Human Anti-HCoV NL63 Virus Spike 1 [S1] IgM ELISA Kit is an immunoassay suitable for quantifying IgM antibody activity specific for S1 subunit of the spike protein of the HCoV NL63 virus, etiologic agent for the COVID-19 respiratory disease, in serum or plasma of vaccinated, immunized and/or infected hosts.

This immunoassay is suitable for:

o Determining immune status relative to non-immune controls;

o Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization, and timing;

o Qualifying and standardizing vaccine batches & protocols.

The assay is for research use only (RUO) and is not intended nor validated for diagnosing HCoV NL63 virus disease. Reagents contain no virus or viral antigens.

General Description

Coronaviruses are a group of highly diverse RNA virus in the Coronaviridae family that are divided in 4 genera: alpha, beta, gamma and delta that cause disease varying from mild to severe in human and animals. Coronaviruses endemic to humans include the alphacoronavirus 229E and NL63 and betacoronaviruses OC43 and HKU1 that can cause influenza-like illness or pneumonia in humans. The genome of the coronavirus encodes 23 putative proteins including 4 major structural proteins: nucleocapsid [N protein], spike [S protein], membrane [M] and small envelope proteins [E]. The S protein is a glycoprotein essential for viral attachment to the host cell surface receptors and translocation into the infected cells; trimers of the S protein make up the spikes of the virus. For cell entry, S1 binds to a host receptor for viral attachment, and S2 undergoes dramatic structural changes to fuse the viral and host membranes. The sequences, structures, and membranefusion mechanisms of the S2 subunits are conserved among different coronavirus genera. However, the S1 subunits from different coronavirus genera share little or no significant sequence similarity.

Principles of Testing

The Anti-HCoV NL63 S1 IgA ELISA kits are based on the binding of antibodies (IgA) in samples to the recombinant, purified HCoV NL63 S1 antigen immobilized on the microwells. Bound antibody is detected by anti-human IgA-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the HRP substrate, which is directly proportional to the amount of antiHCoV NL63 S1 IgA present in the sample. Stop Solution is added to terminate the reaction, and absorbance is then measured using an ELISA reader at 450nm. The presence of antibody (IgA) in samples is determined relative to anti-SARS S1 Calibrators.

Reagents And Materials Provided

1. HCoV NL63 S1 Coated Strip Plate: 8-well strips(12). Coated with purified recombinant HCoV NL63 S1, and post-coated with stabilizers.
2. Anti-HCoV NL63 S1 Calibrators: 1 U/ml, 5 U/ml, 5 U/ml, 10 U/ml. 0.65ml for each. Four (4) vials, each

containing anti-HCoV NL63 S1; in buffer with antimicrobial as stabilizers.

3. Anti-HCoV NL63 S1 Positive Control: 0.65 ml. Antiserum with anti-HCoV NL63 S1 activity; [value range on label]
4. Low NSB Sample Diluent (LNSD): 30 ml. Buffer with protein, detergents and antimicrobial. Use as is for sample dilution.
5. Wash Solution Concentrate (100x): 10ml.
6. Sample Diluent Concentrate (20x): 10ml.
7. Anti-Human IgA-HRP Conjugate Concentrate (100x): 0.15ml.
8. TMB Substrate: 12ml. Chromogenic substrate for HRP containing TMB and peroxide.
9. Stop Solution: 12ml. Dilute sulfuric acid.

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml.
2. Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgG HRP Concentrate.
3. Stock bottle to store diluted Wash Solution; 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. Microwell plate reader at 450 nm wavelength and ELISA plate washer

Storage

The microtiter well plate and all other reagents, if unopened, are stable at 2-8 °C until the expiration date printed on the box label.

Specimen Collection And Preparation

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Sample Dilution

Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example:

Initial (1:5): 10ul serum + 40ul WSD [or 0.1ml + 0.4ml]

Further (1:50): 10ul initial (1:5) + 90ul LNSD (1:50)

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

1. Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample and control to be assayed.
2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
3. Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Reagent Preparation

1. Wash Solution Concentrate (100x): 10ml.

Dilute the entire 10 ml volume with 1L distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at refrigerated for long term and ambient temperature for short term.

2. Sample Diluent Concentrate (20x): 10ml.

Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample/Conjugate Diluent and store at 2-8 °C until the kit lot expires or is used up.

3. Anti-Human IgA-HRP Conjugate Concentrate (100x): 0.15ml

Peroxidase conjugated anti-human IgA in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample/Conjugate Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100x to 2-8 °C storage.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1 st Incubation [100ul – 60 min; 4 washes]

o Add 100ul of calibrators, samples and controls each to predetermined wells.

o Tap the plate gently to mix reagents and incubate for 60 minutes.

o Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2 nd Incubation [100ul – 30 min; 5 washes]

o Add 100ul of diluted Anti-Human IgA HRP to each well.

o Incubate for 30 minutes.

o Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

o Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

o Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

o Add 100ul of Stop Solution to each well.

o Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

o Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

o Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

Interpretation Of Results

Method A. Antibody Activity Threshold Index

Compare Samples to 1 U/ml Calibrator or Internal Control =Positive/Negative Cut-off.

Results

The sensitivity of the assay to detect anti-HCoV NL63 S1 IgA, from either natural exposure or vaccination, is controlled so that the 1 U/ml Calibrator represents a threshold OD for most true positives in human serum diluted to 1:500 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-HCoV NL63 S1 antibody, derived from S1 immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – antiserum reactive to HCoV NL63 S1; value range is on the vial label. This Control can be used to assess reproducibility and to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (A,B,C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a Threshold Index that numerically discriminates Positive/Negative:

❖ Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 is Negative for antibody.

Example:

Human Serum IgA

A panel of sera from normal donors of unknown history (pre-2020) was tested for anti-HCoV NL63 S1 IgA (diluted in Low NSB Sample Diluent). Threshold Index was calculated using the 1 U/ml Cal.

Results

Anti-HCoV NL63 S1 IgA: all sera were positive at 1:500 (above the 1.0 threshold) except one (A).

Notes:

1. Positives may be due to prior encounter with the virus or non-HCoV NL63 proteins with common epitopes, from vaccination, or may be an aspect of the innate immune repertoire.
2. When the Positive Index is above 5.0, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The sensitivity of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:2000) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:200) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a Positive Index (see below) or use an Internal Control.

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Nonimmune samples = Positive Index.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 is Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution. This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

C. Antibody Titer

The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading in linear range of dilution curves of each antibody as Index. In the example below, IgA titers were calculated as inverse of the dilution that produced a 1.0 OD in the assay.

Results

Anti-HKU1: normal human serum; Titer: 14.5 k

Anti-OC43: normal human serum; Titer: 9.3 k

Anti-229E: normal human serum; Titer: 3.95 k

Anti-NL63: normal human serum; Titer: 1.75 k

Sensitivity

The HCoV NL63 S1-coated plate, anti-human IgA-HRP concentration, and Low NSB Sample Diluent are optimized to differentiate anti-HCoV NL63 S1 IgA from background (nonantibody) signal with human serum/plasma samples diluted 1:500.

Specificity

Recombinant HCoV NL63 S1 protein, (APF29071.1) 710 aa/ 78.8 kDa, was expressed as His-tag fusion protein in HEK293 cells, purified and coated on microwells; stabilizing postcoat contains BSA. The Anti-Human IgA HRP conjugate is specific for IgA; IgG, IgM and IgE class antibodies would not be detected above

background.

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.