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antibody and ELISA experts

SARS-CoV-2 Human IgG (4-Plex) Multiplex ELISA Kit (EUA Approved)

Catalog number: MEK2009-1

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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SARS-CoV-2 Human IgG (4-Plex) Multiplex ELISA Kit (EUA Approved)

Catalog Number: MEK2009-1
Storage and Shelf Life : Store unopened kit at 2-8°C until kit expiration. Avoid exposure to UV light.

Product Overview

The Q-Plex™ SARS-CoV-2 Human IgG (4-plex) Multiplex ELISA Kit is a qualitative chemiluminescent assay (ELISA) allowing concurrent measurement of human IgG antibodies to SARS-CoV-2 S1 and SARS-CoV-2 S2 proteins in serum and plasma samples. It has been authorized by FDA under a EUA for use by high complexity CLIA labs. Sensitive, reproducible, and convenient 96-well format requiring low sample volumes. Requires the Q-View™ Imaging System for analysis.

Assay Principle

The Q-Plex™ SARS-CoV-2 Human IgG (4-Plex) multiplex assay is a qualitative enzyme-linked immunosorbent assay that detects IgG antibodies to SARS-CoV-2 in human serum or plasma. SARS-CoV-2 is the novel coronavirus that causes COVID-19. The SARS-CoV-2 virus has several structural proteins including two spike proteins, S1 and S2. When an individual is infected with the SARS-CoV-2 virus, their immune system produces antibodies to these viral proteins. The typical immune response produces detectable antibody levels ~8-10 days following the onset of symptoms.

The Q-Plex SARS-CoV-2 Human IgG Quantitative (4-Plex) Multiplex ELISA detects IgG antibodies to both the S1 and S2 spike proteins present in a blood sample. If antibodies are detected, the result will be considered positive. If antibodies are not detected, the result will be considered negative. The assay only detects IgG antibodies, not other classes of antibodies such as IgM or IgA.

The multiplex assay allows for simultaneous indirect ELISA on the following four printed spots:

1. SARS-CoV-2 Spike Glycoprotein (S1), a recombinant antigen which contains amino acids 1-674 of subunit 1. Spike S1 is expressed in mammalian HEK293 cells with a Sheep Fc-Tag.
2. SARS-CoV-2 Spike Glycoprotein (S2) is a recombinant antigen which contains the Spike subunit 2 protein, amino acids 685-1211. Spike S2 is expressed in mammalian HEK293 cells with a Sheep Fc-Tag.
3. Sheep Fc is a negative control to ensure no cross-reactivity occurs between human IgGs in the sample and the Fc-Tag on the SARS-Cov-2 Spike proteins.
4. Anti-Human IgG is a positive control to ensure the kit performs and the IFU was followed correctly.

Assay Validation

The Q-Plex™ SARS-CoV-2 Human IgG (4-Plex) ELISA tests for IgG antibodies to either the SARS-CoV-2 S1 protein or the SARS-CoV-2 S2 protein. Our validation studies identified measurable improvement in clinical performance when results from the S1 and S2 assays are considered together rather than single assays (see assay validation data below). Quansys recommends that a sample should only be considered positive for the presence of SARS-CoV-2 reactive IgGs when antibodies reactive to both S1 and S2 are detected.

Negative samples, collected prior to August 2019, and known positive samples collected from individuals who tested positive for COVID-19 on a molecular test at least 14 days prior to sample collection were used to determine clinical sensitivity and specificity.

Evaluating the S1 and S2 simultaneously allows for greater clinical sensitivity and specificity.

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SARS-CoV-2 IgG Assay (Combined Result from S1 & S2) vs. Molecular COVID-19 Test

N = 576	Confirmed Positive	Confirmed Negative
IgG Test Positive	33	0
IgG Test Negative	1	542

The combined S1 & S2 reactive antibody assay demonstrates:

Estimated Sensitivity	(PPA)	97%
Estimated Specificity	(NPA)	100%
	PPV	100%
	NPV	100%

SARS-CoV-2 IgG Assay Characteristics

Principle	Indirect ELISA
Sample Type	Human Serum, Plasma, Whole Blood
Sample Volume	2 µL
Assay Incubation	2 hours, RT
Total Wash Steps	3
Within Plate Reproducibility	8% CV
Between Plate Reproducibility	10% CV

Assay Ranges

Analyte	Assay Type	Calibrator Range	Upper Limit of Quantification (ULOQ)	Lower Limit of Quantification (LLOQ)	Limit of Detection	Precision (Inter-assay)	Precision (Intra-assay)	Average Linearity
SARS-CoV-2 S1	Indirect	1,000 - 1.37 (U/mL)	700 (U/mL)	5.50 (U/mL)	0.69 (U/mL)	10%	9%	107%
SARS-CoV-2 S2	Indirect	1,000 - 1.37 (U/mL)	700 (U/mL)	5.50 (U/mL)	0.69 (U/mL)	12%	9%	104%

Kit Components

Each kit contains a 96-well plate, featuring the relevant biomarker panel in each well, and all reagents required to perform testing.

Reagents include:

Wash Buffer Concentrate (20X): Liquid, 50 mL/vial of a concentrated solution of buffered surfactant
Sample Diluent (2X): Liquid, 10 mL/vial of a concentrated buffered protein solution with preservatives
Detection Mix: Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives
Calibrators (High, Low, Negative): Lyophilized, human serum diluted in a buffered protein base
Streptavidin-HRP (1X): Liquid, 6 mL/vial of streptavidin-conjugated horseradish peroxidase
Substrate A: Liquid, 3 mL/vial of peroxide solution
Substrate B+: Liquid, 3 mL/vial of luminol solution
Plate Seals (3): Adhesive strips

Kit Processing

The Multiplex assays require the use of the Q-View™ software to read and interpret the test results. A fully-functional, free trial version of the software is available to download, install, and use to analyze your first kit (s). At the end of the trial period, a purchased license is required to continue the use of the Q-View software.

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Q-Plex arrays are developed and optimized to work with Q-View imagers. We do not guarantee the results obtained from other imagers because not all imagers are compatible with Q-Plex arrays.

Assay Principle

This multiplex assay is based on the 96 well plate sandwich enzyme immunoassay technique for the measurement of multiple analytes. For each analyte, two different antibodies targetting different epitope are used, one arrayed to the bottom of the plate and one for detection of the captured analytes. Samples or calibrators are pipetted into wells of a 96 well plate and the analytes of interests are immobilized by their corresponding capture antibodies. After washing away any unbound proteins, a mixture that contains biotinylated analyte specific antibodies is added. The biotinylated antibodies complete the sandwich for each specific arrayed analyte. After washing away unbound biotinylated antibody, streptavidin-horseradish peroxidase (SHRP) is added. Following an additional wash, the amount of SHRP remaining on each location of the array is proportional to the amount of analytes initially captured. The amount of conjugated enzyme on each location of the array is measured with the addition of a chemiluminescent substrate.

Sample Collection And Storage

The sample collection and storage conditions are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Cell culture supernates should contain at least 1% fetal calf serum for stability of the proteins. Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Before Starting

1. Read all instructions before beginning test.

2. For research use only. Not for use in diagnostic procedures.

3. The kit should not be used beyond the expiration date on the kit label.

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4. **This kit is sensitive to saliva. Wear a mask during preparation and running the kit.**

5. If running multiple kits, a calibration curve must be included for each 96-well plate. The curve from one plate cannot be used to calculate sample values from other plates.

6. Do not mix or substitute reagents with those from other kits or lots.

7. All products are carefully validated, however due to the variability encountered in biological buffers and sample matrices, the possibility of interference or sample matrix effects cannot be excluded.

8. Warning: The calibrator contains components of human origin. These components have been tested at the donor level and found negative for HBsAg, HIV-1 and HIV-2 antibodies, and HCV. However, consider all materials as potentially infectious and use only approved guidelines for the proper handling and disposal of infectious material.

Best Practices

Dilute all samples at least 1:2 (one part sample to one part diluent) with the provided sample diluent to prevent false positives, and mix thoroughly.

Load all calibrators and samples into the microplate within 10 minutes of each other.

Be exact with incubation times, particularly the streptavidinhorseradish peroxidase (SHRP) incubation.

Be exact when mixing Substrate A and B and mix thoroughly.

Do not allow the plate to dry out between steps.

Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade it.

Materials Needed But Not Included

1. Multichannel pipette (20-200 uL) and/or a single channel pipette (20- 200 uL) and tip

2. Polypropylene tubes or polypropylene 96-well plate(s) for sample and calibrator preparation

3. Microplate shaker capable of 500 RPM.

4. Deionized water

5. Microplate washer

6. Graduated cylinder for the preparation of wash buffer

Assay Preparation

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1. Set the plate shaker to 500 RPM.

2. Prepare the Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water, and mix thoroughly.

3. Prepare Calibrator: Reconstitute using Sample Diluent with the volume on the Product Card which accompanies the kit. Allow Calibrator to sit for 5 minutes. Mix thoroughly.

4. Allow Substrate A and B to come to room temperature (20-25°C). Fifteen minutes prior to use, combine 3 mL of Substrate A with 3 mL of Substrate B, and mix gently. Do not expose to UV light. Store at room temperature (20-25°C) after mixing.

Assay Procedure

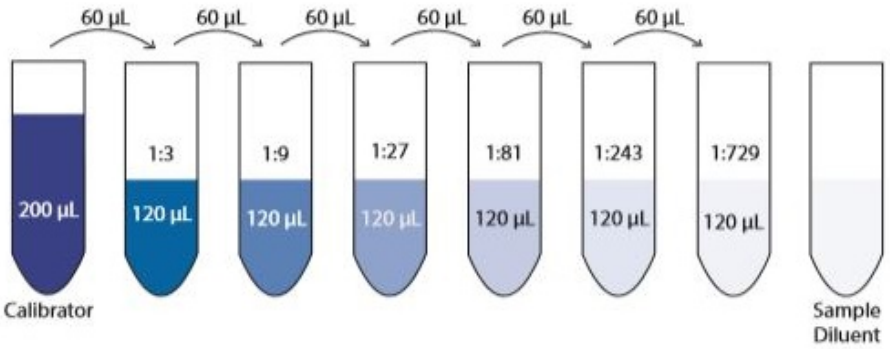
This assay is saliva sensitive. Wear a mask when running this kit. Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed by the previous sections. It is recommended that all calibrators, samples, and controls be assayed in duplicate.

1. Using the previously reconstituted calibrator, prepare an 8-point calibration curve (7 points plus 1 blank) in either polypropylene tubes or a polypropylene 96-well plate.

a. Pipette 200 uL of prepared calibrator into the first tube or well.

b. Place 120 uL of prepared sample diluent into the other seven tubes or wells.

c. Transfer 60 uL of the undiluted prepared calibrator from the first tube into the second, mix thoroughly, and repeat the transfer from tube to tube for 5 more points, leaving the last tube without any prepared calibrator. This process is diagrammed above. The undiluted prepared calibrator serves as the high point of the standard curve. The prepared sample diluent serves as the negative.



2. Prepare plasma samples by diluting 1:2 (one part sample to one part prepared sample diluent) with enough prepared sample diluent to have 50 uL per well in either polypropylene tubes or a polypropylene 96-well plate.

Note: If you anticipate that your analyte concentrations will be greater than the high point of the calibration curves as reported on the product card included in the kit, use the prepared sample diluent to dilute your samples further.

3. Add 50 uL per well of the calibration curve and samples by pre-wetting the pipette tips three times, drawing up the liquid into your pipette and then dispensing back into the original vessel, aspirating 50 uL and dispensing into the 96-well plate. Load all samples and calibration curve to the

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plate within ten minutes.

4. Cover the plate with a plate seal provided, and shake on a plate shaker set to 500 RPM for one hour at room temperature (20-25°C).

5. Wash the plate three times

6. Add 50 uL per well of Detection Mix, cover with a new plate seal, and return to the plate shaker set to 500 RPM for one hour at room temperature (20-25°C).

7. Wash the plate three times (see Appendix A).

8. Add 50 uL per well of Streptavidin-HRP 1X, cover with a new plate seal, and return to the plate shaker set to 500 RPM for 15 minutes at room temperature (20-25°C).

9. Wash the plate six times (see Appendix A).

10. Add 50uL per well of previously prepared substrate. Wait no longer than 15 minutes to commence imaging. Note: If imaging cannot commence immediately, protect the plate from drying for up to 15 minutes by dispensing 100 uL of wash buffer into each well of the plate prior to adding the mixed substrate. When ready to image, remove the wash buffer from the plate and add the substrate.

11. Set aperture (f-stop) to the lowest value to a minimum of 2, set binning to the lowest value.

12. Adjust zoom so the plate takes up about 80% of the field of view in the imaging software.

13. Adjust focus so that individual spots on the Calibration Kit mouse pad are clear and visible in the imaging software.

14. Place the plate in the compatible imager, take image and export result in grayscale images using “Export For Analysis”, “Export Raw Data,” etc. Choose a lossless file type, such as TIFF and at least 12-bit depth output.

15. Dispose of all used and unused materials. Disposal of waste may differ from country to country. Please refer to local disposal rules.

16. Send the result data to support@bosterbio.com for analysis. If you would like to analyze the data internally, please contact us to set up.

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