QUANSYS BIOSCIENCES

Q-Plex™ SARS-CoV-2 Human IgG (4-Plex)

In vitro diagnostic use. For prescription use only.
For emergency use authorization only. Version 2.9.9 10/2020

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Symbol	Explanation
REF	Catalog Number
LOT	Lot Number
	Use By YYYY-MM-DD
	Temperature Limitation
	Manufacturer
	Keep Away From Sunlight
IVD	In vitro diagnostic medical device

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NAME AND INTENDED USE

Q-Plex™ SARS-CoV-2 Human IgG (4-Plex)
Quansys Biosciences Catalog Number 691649HU

The Q-Plex SARS-CoV-2 Human IgG (4-Plex) is an Enzyme-Linked Immunosorbent Assay (ELISA) intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (dipotassium EDTA and lithium heparin). The Q-Plex SARS-CoV-2 Human IgG (4-Plex) is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection.

At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Q-Plex SARS-CoV-2 Human IgG (4-Plex) should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a, that meet requirements to perform high complexity tests.

Results are for the detection of SARS-CoV-2 antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of the Q-Plex SARS-CoV-2 Human IgG (4-Plex) early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the Q-Plex SARS-CoV-2 Human IgG (4-Plex) may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using a second, different IgG assay.

The Q-Plex SARS-CoV-2 Human IgG (4-Plex) is only for use under the Food and Drug Administration's Emergency Use Authorization.

PRINCIPLE OF THE ASSAY

This assay is based on the microplate indirect enzyme immunoassay technique for the detection of human IgG antibodies reactive to SARS-CoV-2 (SARS-CoV-2 S1 and SARS-CoV-2 S2).

The microplate is arrayed with 4 spots in each well:

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), 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, a negative control to ensure no cross-reactivity occurs between human IgG in the sample and the Fc-Tag on the SARS-CoV-2 Spike proteins.
4. Anti-human IgG, a positive control to ensure the kit IFU was followed correctly and that human IgG is present in the sample.

Samples, calibrators, or external controls are pipetted into wells of an arrayed microplate thereby immobilizing antibodies to SARS-CoV-2 S1, SARS-CoV-2 S2, Sheep Fc (negative control), and anti-human IgG (positive control) to their locations in the array. After washing away any unbound material, a mixture that contains biotinylated anti-human IgG is added. After washing away unbound biotinylated antibody, streptavidin-horseradish peroxidase (SHRP) is added. The last step is addition of a chemiluminescent substrate and the plate is read using the Q-View™ Imager Pro or Q-View™ Imager LS and Q-View™ Software.

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions are intended as general guidelines. The Clinical and Laboratory Standards Institute (CLSI GP44-A4) recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C. If the assay will not be completed within 48 hours, or if the samples will be stored beyond 48 hours, samples should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed. Frozen samples must be mixed well after thawing and prior to testing. Samples prepared in Sample Diluent should be tested within 8 hours. Do not use bacterially contaminated samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine its own specific stability criteria.

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 K2 EDTA or lithium 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.

QUALITY CONTROL

Each microplate well includes the following immobilized control protein spots:

1. The sheep Fc spot is a negative control to ensure no cross-reactivity occurs between human IgG antibodies in the sample and the Fc-Tag on the SARS-CoV-2 Spike proteins. For all samples and controls, the within-well Sheep Fc spot signal is subtracted from the SARS-CoV-2 S1 and SARS-CoV-2 S2 signals to create corrected S1 and S2 signals.

2. The anti-human IgG spot is a positive control to ensure the kit IFU was followed correctly. The anti-human IgG positive control detects human IgG present in a sample. In the case of failure to load an acceptable sample, the anti-human IgG spot will produce low signal and the Q-View software will report an error code in the Report tab for that sample. The results from a well with such an error code are invalid; the sample contained within that well should be rerun. The lack of an error code indicates that the sample was prepared and loaded correctly.

In addition, the following calibrators are provided with the test kit:

1. High and Low positive calibrators. High and Low positive calibrators are provided with the kit to be tested in duplicate on every 96-well ELISA plate. The High and Low positive calibrators are lyophilized human serum that have been characterized as positive for SARS-CoV-2 IgG. The calibrators are used to determine the cutoff values for the S1 and S2 protein spots by dividing the average corrected signals of the Low Calibrator by the High Calibrator for the respective spots and multiplying by the lot specific Lot Cutoff Correction Value for each spot. Then S1 and S2 Sample Ratios are calculated for each sample by dividing corrected sample values of S1 and S2 by the average corrected High Calibrator value.

2. A negative calibrator is provided with the kit to be tested on every 96-well ELISA plate. The negative calibrator is needed to determine the validity of plate results. The calibrator is a lyophilized human serum that has been characterized as negative for SARS-CoV-2 IgG. For a test to be valid, the ratio of the Low Calibrator to the Negative Serum calibrator must be at least 3 for the S1 spot. If this condition is not met, the results from the entire plate are not valid.

The test kit does not contain external controls:

1. External controls are available for separate purchase from Seracare (Milford, MA), ACCURUN® Anti-SARS-CoV-2 Reference Material Kit – Series 1000, containing both positive and negative controls. The negative and positive controls are intended to monitor for substantial reagent failure. The test is invalid and must be repeated if the control samples do not return a negative (reactive IgG antibodies not present) or positive (reactive IgG antibodies present) result. If the test is invalid, the sample results cannot be used and should be retested. The positive material is manufactured from human serum or plasma reactive for SARS-CoV-2 IgG and nonreactive for HBsAg and antibodies to HIV 1 and 2, HTLV I and II, and HCV. The negative reference material is manufactured from human serum or plasma nonreactive for antibodies to SARS-CoV-2, as well as HBsAg and antibodies to HIV 1 and 2, HTLV I and II, and HCV.

It is recommended to test controls in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control procedures.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use
2. For Emergency Use Authorization only.
3. This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform high complexity tests.
4. This test has been authorized only for the presence of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens.
5. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food Drug and Cosmetic Act, 21 U.S.C. § 360bbb3(b)(1), unless the authorization is terminated or revoked sooner.
6. Read all instructions before beginning test.
7. The kit should not be used beyond the expiration date on the kit label.
8. If running multiple kits, calibrator samples must be included for each 96-well plate. The calibrator values from one plate cannot be used to determine sample results from other plates.
9. Do not mix or substitute reagents with those from other kits or lots.
10. Pre-wet pipette tips three times by drawing up the liquid into the pipette and then dispensing back into the original vessel prior to the addition of samples, calibrators, or external controls to the microplate.
11. Load all external controls, calibrators and samples into the microplate within 5 minutes of each other.
12. Be exact with incubation times, particularly the streptavidin-horseradish peroxidase (SHRP) incubation.
13. Be exact when mixing Substrate A and B+ and mix thoroughly.
14. Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade them.

15. Do not allow the plate to dry out between steps.
16. Warning: The calibrators contain components of human origin. These components have been heat inactivated. However, consider all materials as potentially infectious and use only approved guidelines for the proper handling and disposal of infectious material.

KIT CONTENTS & STORAGE

Unopened Kit -  Store at 2-8°C. Do not use kit past expiration date. Do not re-use the kit.

Part/Description	Storage of opened/reconstituted material
Q-Plex™ Array Microplate One arrayed and blocked 96-well polystyrene microtiter plate	 2-8°C until kit expiration
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	 2-8°C until kit expiration Discard unused reconstituted calibrators.
Streptavidin-HRP 1X Liquid, 6 mL/vial of streptavidin-conjugated horse-radish peroxidase	 Do not expose to UV light. 2-8°C until kit expiration
Substrate A Liquid, 3 mL/vial of peroxide solution	 Do not expose to UV light. Store mixed substrate solution at room temperature (20-25°C) for up to 1 week. Store unmixed solutions at 2-8°C until kit expiration.
Substrate B+ Liquid, 3 mL/vial of luminol solution	
Plate Seals (3) Adhesive strips	Non-perishable

MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to the kit contents listed, the following materials are required to run this assay.

1. Multichannel pipette (20-200 µL) and/or single channel pipettes (2 - 1000 µL) with appropriate tips
2. Polypropylene tubes or polypropylene 96-well plate(s) for sample, calibrator, and external control preparation
3. Q-View Imager Pro or Q-View Imager LS and Q-View Software
4. Deionized water
5. Microplate washer (An optional multichannel pipette protocol is provided in Appendix A.)
6. Graduated cylinder for the preparation of wash buffer and storage container
7. 50 mL conical tube or other container for diluting the 2X Sample Diluent

ASSAY PREPARATION

1. Install the Q-View Software on the computers that will be used for analysis or operating a Q-View Imager Pro or Q-View Imager LS.
2. Set up the imager. For imager-specific instructions, see www.quansysbio.com/manuals.
3. Prepare Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water and mix thoroughly to make 1X Wash Buffer.
4. Prepare Sample Diluent: Dilute the 2X Sample Diluent into 10 mL of deionized water and mix thoroughly to make 20 mL of 1X Sample Diluent
5. Prepare Calibrators: Reconstitute using 1X Sample Diluent with the volume on the Product Card which accompanies the kit. Allow Calibrators to sit for 5 minutes. Mix thoroughly.
6. 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

Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed by the previous sections. It is recommended calibrators be assayed in duplicate.

1. Dilute samples 1:100 (one-part sample to ninety-nine parts prepared sample diluent) in either polypropylene tubes or a polypropylene 96-well plate in a volume sufficient to provide 50 µL of diluted sample per well.
2. Add 50 µL per well of the each of the calibrators to duplicate wells of the microarrayed plate.
3. Add 50 µL per well of diluted samples to either single or duplicate wells. Load all samples and calibrators to the plate within five minutes.
4. Cover the plate with a plate seal provided and incubate for one hour at room temperature (20-25°C).
5. Wash the plate three times (see Appendix A).
6. Add 50 µL per well of Detection Mix, cover with a new plate seal, and incubate for thirty minutes at room temperature (20-25°C).
7. Wash the plate three times (see Appendix A).
8. Add 50 µL per well of Streptavidin-HRP 1X, cover with a new plate seal, and incubate for twenty minutes at room temperature (20-25°C).
9. Wash the plate six times (see Appendix A).
10. Add 50 µL per well of previously prepared substrate. Image plate immediately. Wait no longer than 5 minutes to commence imaging.

    Note: If imaging cannot commence immediately, protect the plate from drying for up to 15 minutes by dispensing 100 µL 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. Place the plate in the Q-View Imager Pro or Q-View Imager LS.
12. Open Q-View Software, create or open a project, and click Acquire Image.

13. When using a Q-View Imager Pro, set the exposure time to 300 seconds.
14. When using a Q-View Imager LS, set the exposure time to 270 seconds.
15. Click the Capture Image(s) button. Users may continue on to Well Assignment while images are being captured.

    Note: Details about these imaging steps are available in the Q-View Software Manual viewable at www.quansysbio.com/manuals or within Q-View Software under **Support > Manual**.

Dispose of all used and unused materials. Disposal of the potentially hazardous waste may differ regionally. Please refer to local disposal rules.

ANALYZING A Q-PLEX™ IMAGE

The following summarizes a general workflow for analyzing a Q-Plex image in Q-View Software. Each of these steps is described in greater detail in the Q-View Software and Imager Manual, viewable at www.quansysbio.com/manuals or within Q-View Software under **Support > Manual**.

1. Acquire or import an image into Q-View Software as described above.
2. Enter the **Product Code** (found on the Product Card) into the **Product Code** field.
3. **Image Processing:** Align the plate overlay as follows:
    a. Set the overlay. If using the **Auto-Set Plate Overlay** feature, this will occur automatically. Otherwise, go to **Overlay Options > Set Plate Overlay**
    b. To visualize bright or dim spots, optimize the display using **Image Options > Adjust Gamma** (does not affect the data).
    c. Optimize overlay alignment: Go to **Overlay Options > Adjust Plate Overlay** to pivot the overlay, **Adjust Well** and **Adjust Spot** to move individual wells and spots, then **Auto-Adjust Spots** to automatically snap each circle of the overlay to the nearest spot of the image beneath.
4. **Well Assignment:** Label wells as samples, calibrators, or controls. Use **Templates** to quickly assign layouts that are repeated often or export the layout as a .csv file.
5. **Data Analysis:** Once Image Processing and Well Assignment are complete, select Data Analysis. Click Perform Analysis to generate charts and statistics. For this qualitative assay, data analysis must be done on signal, which can be viewed on the Report tab and selected from the Statistics menu as “Pixel Intensity”.

INTERPRETATION OF RESULTS

The assay uses a calculated cutoff value (explained below) to determine positive and negative results.

1. **Q-View Software Checks:** Q-View Software provides checks to ensure the validity of the result.

    a. The anti-human IgG spot detects human IgG present in a sample. In the case of failure to load an acceptable sample, the anti-human IgG spot will produce low signal. In this case, Q-View software will report an error code (AC4) in the Report tab for that sample. The results from a well with such an error code are invalid.

    b. The software also determines the presence of non-specific well background at several locations within the well. Where non-specific background is present at unacceptable levels, the software will report an error code (AC5, AC6, AC7, or AC8) in the Report tab for that sample. If two or more of these error codes are present for a given well, the results from that well are invalid.

    c. The user should review any error codes reported by Q-View software.

Note: The following steps are conducted manually by the user. See Appendix B for a worksheet calculation aid for steps 2b through 2h.

2. **Determine the validity of the test:** For plate results to be valid, the ratio of the Low Calibrator to the Negative Calibrator must be at least 3 for the S1 spot. If this condition is not met, the results from the entire plate are not valid.

    a. Average Signal of the Low Calibrator/Average Signal of the Negative Serum Calibrator ≥ 3.

    b. Correct S1 and S2 signal: For all samples, calibrators and controls, subtract the within-well Sheep Fc spot signal (spot 3) from SARS-CoV-2 S1 (spot 1) and SARS-CoV-2 S2 (spot 2) signals to create the corrected S1 and S2 signals. If the result is <0, replace result with 0.

c. Calculate the average corrected High Calibrator S1 and S2 signal.

d. Calculate the average corrected Low Calibrator S1 and S2 signal.

e. Determine the S1 and S2 Cutoff Values: Calculate the Calibrator Ratios for S1 and S2 by dividing their respective average corrected Low Calibrator signal by their average corrected High Calibrator signal.

f. Multiply the Calibrator Ratio by the Lot Cutoff Correction Value on the Product Card. These adjusted values are the Cutoff Values for the S1 and S2 spots.

g. Determine the S1 and S2 sample specific ratios: Calculate the S1 and S2 Sample Ratio of each sample by dividing corrected sample value (step c.) by the average corrected High Calibrator (step d.) and compare to the S1 and S2 Cutoff Values (step g.). Samples with ratios on S1 that are higher than the S1 cutoff indicate IgG antibody reactivity to the Spike 1 protein spot. Samples with ratios on S2 that are higher than the S2 cutoff indicate IgG antibody reactivity to the Spike 2 protein spot.

h. Only samples that are positive for both the S1 and S2 spots are considered to have reactive antibodies to SARS-CoV-2 (See Table 1).

Table 1: Results and Reporting

Result			
SARS-CoV-2 S1	SARS-CoV-2 S2	Final Interpretation	Reporting Instructions
Sample ratio > cutoff	Sample ratio ≤ cutoff	Non-Reactive	Report result as non-reactive for SARS-CoV-2 IgG antibodies
Sample ratio ≤ cutoff	Sample ratio > cutoff	Non-Reactive	Report result as non-reactive for SARS-CoV-2 IgG antibodies
Sample ratio ≤ cutoff	Sample ratio ≤ cutoff	Non-Reactive	Report result as non-reactive for SARS-CoV-2 IgG antibodies
Sample ratio > cutoff	Sample ratio > cutoff	Reactive	Report result as reactive for SARS-CoV-2 IgG antibodies

3. Data Interpretation Example:

Table 2: Example data

Sample		S1 Signal	S2 Signal	Sheep Fc Signal
High Calibrator	Replicate 1	49182	50307	1480
	Replicate 2	48320	49799	1288
Low Calibrator	Replicate 1	3684	14893	467
	Replicate 2	3540	14309	449
Negative Serum Calibrator	Replicate 1	279	1312	1586
	Replicate 2	269	1260	1524
Sample A	Single Replicate	302	780	1025
Sample B	Single Replicate	15704	6151	1282
Sample C	Single Replicate	35824	15623	985

Determine the validity of the test:

- Average Signal of the Low Calibrator / Average Signal of the Negative Calibrator = (3612/274) = 13.1 ≥ 3. The test is valid.

Correct S1 and S2 signal: Subtract within well Sheep Fc signal to calculate corrected S1 and S2 signal.

- Example: S1 High Calibrator Replicate 1 (49182) – Sheep Fc High Calibrator (1480) = 47702

Table 3: Example data with Sheep Fc subtraction

Sample		Corrected S1 Signal	Corrected S2 Signal
High Calibrator	Replicate 1	47702	48827
	Replicate 2	47032	48511
Low Calibrator	Replicate 1	3217	14426
	Replicate 2	3091	13860
Negative Calibrator	Replicate 1	0	0
	Replicate 2	0	0
Sample A	Single replicate	0	0
Sample B	Single replicate	14422	4869
Sample C	Single replicate	34839	14638

Calculate the average of all replicate data

Table 4: Example data with average replicate data

Sample	Average Corrected S1 Signal	Average Corrected S2 Signal
High Calibrator	47367	48669
Low Calibrator	3154	14143
Negative Serum Calibrator	0	0
Sample A	0	0
Sample B	14422	4869
Sample C	34839	14638

Determine the S1 and S2 Cutoff Values:

- Ratio of the Low Calibrator to the High Calibrator for S1 = 3154/47367 = 0.067
- Ratio of the Low Calibrator to the High Calibrator for S2 = 14143/48669 = 0.29
- Multiply the ratio of the Low Calibrator to the High Calibrator by the Lot Correction Factor: 0.067 * 0.75 = 0.050. This is the cutoff for the S1 spot.
- Multiply the ratio of the Low Calibrator to the High Calibrator by the Lot Correction Factor: 0.29 * 0.41 = 0.12. This is the cutoff for the S2 spot.

Determine the S1 and S2 sample specific ratios:

Table 5: Example sample ratio calculation

	S1 Sample Specific Ratio	S2 Sample Specific Ratio
Sample A	0	0
Sample B	0.30	0.10
Sample C	0.74	0.30

Sample A is negative on the S1 spot: 0 < 0.050.

Sample A is negative on the S2 spot: 0 < 0.12.

Sample A is NEGATIVE.

Sample B is positive on the S1 spot: 0.30 > 0.050.

Sample B is negative on the S2 spot: 0.10 < 0.12.

Sample B is NEGATIVE.

Sample C is positive on the S1 spot: 0.74>0.050.

Sample C is positive on the S2 spot: 0.30>0.12.

Sample C is POSITIVE.

Note: See Appendix B for a worksheet calculation aid.

LIMITATIONS OF THE PROCEDURE

1. For prescription use only
2. For in vitro diagnostic use only
3. For Emergency Use Authorization Only
4. Do not use grossly hemolyzed or lipemic samples.
5. Performance has only been established with the specimens listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
6. This test is for qualitative detection of anti-SARS-CoV-2 IgG antibody in human serum or plasma and does not measure the quantity of the antibodies.
7. Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection or to inform infection status. A molecular assay should be used to evaluate symptomatic patients for acute COVID-19.
8. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.
9. False positive may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings.
10. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
11. Results are not intended to be used as the sole basis for patient management decisions. Test results should be interpreted in conjunction with clinical observations, patient history, epidemiological information, and other laboratory findings.

12. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
13. It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
14. This test should not be used for blood donor screening.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The Q-Plex SARS-CoV-2 Human IgG (4-Plex) Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the Q-Plex SARS-CoV-2 Human IgG (4-Plex), the relevant Conditions of Authorization are listed below:

1. Authorized laboratories[1] using the Q-Plex SARS-CoV-2 Human IgG (4-Plex) will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories will use the Q-Plex SARS-CoV-2 Human IgG (4-Plex) as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instrument, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Q-Plex SARS-CoV-2 Human IgG (4-Plex) are not permitted.
3. Authorized laboratories that receive the Q-Plex SARS-CoV-2 Human IgG (4-Plex) will notify the relevant public health authorities of their intent to run the Q-Plex SARS-CoV-2 Human IgG (4-Plex) prior to initiating testing.
4. Authorized laboratories using the Q-Plex SARS-CoV-2 Human IgG (4-Plex) will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

1 The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

5. Authorized laboratories will collect information on the performance of the Q-Plex SARS-CoV-2 Human IgG (4-Plex) and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quansys Biosciences (ccall@quansysbio.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the Q-Plex SARS-CoV-2 Human IgG (4-Plex) of which they become aware.

6. All laboratory personnel using the Q-Plex SARS-CoV-2 Human IgG (4-Plex) must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the Q-Plex SARS-CoV-2 Human IgG (4-Plex) in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product.

7. Quansys Biosciences, authorized distributors, and authorized laboratories using the Q-Plex SARS-CoV-2 Human IgG (4-Plex) will ensure that any records associated with the EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

PERFORMANCE CHARACTERISTICS

Class Specificity

The anti-human IgG detection antibody used in the Q-Plex SARS-CoV-2 Human IgG (4-Plex) assay demonstrates class-specific reactivity to human IgG isotypes. No binding interactions were observed to human IgA, human IgE, human IgD, and less than 1% cross reactivity was observed with human IgM at the tested concentrations.

Biotin Interference Study

A study was performed to evaluate the potential interference of biotin.

Five SARS-CoV-2 IgG positive samples and five SARS-CoV-2 IgG negative samples were selected and spiked with biotin at 3500, 1750, 875, 438, 219, and 0 ng/ml.

All samples were tested in duplicate. All duplicate tests had the same result. Sample results below (Table 6) include each sample at each spike level, not including duplicate results.

Table 6: Biotin Interference Study Results

Clinical Call		
SARS-CoV-2 IgG Assay vs. Molecular COVID-19 Test		
N=60	Confirmed Positive	Confirmed Negative
IgG Test Positive	30	0
IgG Test Negative	0	30
PPA	100%	
NPA	100%	

All positive samples spiked with any level of biotin up to 3500 ng/ml had a positive result. The PPA is 100%. All negative samples spiked with any level of biotin returned a negative result. The NPA is 100%.

High biotin levels in samples do not interfere with the Q-Plex SARS-CoV-2 Human IgG (4-Plex) test.

Clinical Performance

Six hundred and twenty-four (624) clinical serum and plasma samples (585 negative samples collected prior to August 2019 or from subjects with known negative RT-PCR results, and 39 samples collected from subjects with known positive RT-PCR results) collected by a vendor from U.S. patients were tested on the Q-plex SARS-CoV-2 Human IgG (4-Plex) assay to evaluate clinical agreement. The positive percent agreement (PPA) was 97.4%, 38/39, (95% CI: 86.8 – 99.5) and the negative percent agreement (NPA) was 99.7%, 583/585, (95% CI: 98.8 – 99.9). The details of these results are shown in the tables 7-9 below.

Table 7: Clinical Agreement Sample Profile

Sample	COVID19 Positive	COVID19 Negative	Total Number of Samples
Serum Samples collected prior to August 2019	0	336	336
EDTA Plasma Samples collected prior to August 2019	0	213	213
Heparin Plasma Samples collected prior to August 2019	0	26	26
Contemporary Li-Heparin Plasma Samples Collected with known results for molecular test for SARS-CoV-2	18	10	28
Contemporary Serum Collected with known results for molecular test for SARS-CoV-2	21	0	21
Total	**39**	**585**	**624**

Table 8: Q-Plex SARS-CoV-2 Human IgG (4-Plex) assay positive percent agreement (PPA) stratified by days post PCR test

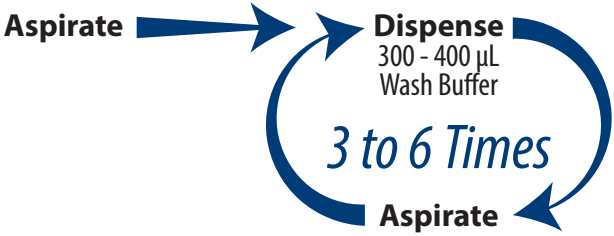
Days post PCR test	Number of Samples Tested	IgG Positive Results	IgG PPA	95% Confidence Intervals
0-7 days	4	4	100%	51.0%-100%
8-14 days	13	13	100%	77.2%-100%
>14 days	22	21	95.2%	77.3%-99.2%

Table 9: Q-Plex SARS-CoV-2 Human IgG (4-Plex) assay negative percent agreement (NPA)

Number of Samples Tested	IgG Negative Results	IgG NPA	IgG NPA 95% Confidence Intervals
585	583	99.70%	98.8%-99.9%

APPENDIX A: PLATE WASHING METHODS

1. Use a program that will aspirate and dispense 300-400 µL wash buffer.



2. Ensure that the plate washer has a program that will not scratch the bottom of the microplate but will prevent plate drying. This is critical to prevent damage to the capture antibody arrays. The simplest method to avoid plate drying is to leave a small, uniform amount (1-3 µL) of wash in the well after the final aspiration, and add the next reagent to the plate as quickly as possible.

3. Leaving a uniform amount of wash in the wells can be accomplished with an automated washer by slightly increasing the aspiration height of the washer head.

   For example:

Process	**Distance**	**Steps on a Biotek ELX-405**
Aspiration Height	3.81 mm	30
Aspiration Position	1.28 mm from center	-28
Dispense Height	15.24 mm	120
No soak or shake cycles are needed		

4. Connect the prepared wash buffer to your automatic plate washer.
5. Run 1-2 priming cycles to make sure that the wash buffer is running through the plate washer. When the buffer has run through the machine, the waste will be foamy.
6. In a spare microtiter plate, dispense 100 µL wash buffer, ensure that all pins dispense uniformly, then aspirate and ensure that all pins aspirate uniformly. This will ensure that all pins are functioning.
7. Prime the plate washer one time before the first wash step. When running the assay, perform the wash 3 or 6 times according to the protocol.

Multichannel Pipette Wash Method

1. Just prior to washing, pour the prepared wash buffer into a trough or tray.
2. After each incubation, flick the solution out of the plate over a waste container before starting the wash protocol.
3. Using a multichannel pipette, dispense 400 µL of wash buffer into each of the wells used in the test.
4. Aggressively flick the wash buffer out over a waste container.
5. This washes the plate one time. When the assay procedure calls for three or six washes, repeat steps 3-4 accordingly.
6. Tap the plate upside down on a paper towel to remove any residual wash.
7. Proceed immediately to dispense the next solution so drying does not occur.

APPENDIX B: WORKSHEET CALCULATION AID

Q-Plex SARS-CoV-2 Human IgG (4-Plex) Calculation Worksheet

Step 1: Correct S1 and S2 Signal

	Data Input			Calculations			
	S1 Signal	S2 Signal	Sheep Fc Signal	Corrected S1 Signal (S1 Signal - Sheep Fc Signal)	Corrected S2 Signal (S2 Signal - Sheep Fc Signal)	Average S1 Signal	Average S2 Signal
High Calibrator Replicate 1							
High Calibrator Replicate 2							
Low Calibrator Replicate 1							
Low Calibrator Replicate 2							
Sample 1							
Sample 2							

Step 2: Determine the S1 and S2 Cutoff Values

	Data Input				Calculations			
	Avg. S1 Signal	Avg. S2 Signal	Lot Cutoff Correction Value for S1 *See Product Card*	Lot Cutoff Correction Value for S2 *See Product Card*	S1 Calibrator Ratio (Average S1 Signal from Low Calibrator / Average S1 Signal from High Calibrator)	S1 Calibrator Ratio (Average S1 Signal from Low Calibrator / Average S2 Signal from High Calibrator)	S1 Cutoff Value (S1 Calibrator Ratio * S1 Lot Cutoff Correction Value)	S2 Cutoff Value (S2 Calibrator Ratio * S2 Lot Cutoff Correction Value)
High Calibrator								
Low Calibrator								

Step 3: Determine the S1 an S2 Sample Specific Ratios

	Data Input				Calculations	
	Avg. S1 Signal	Avg. S2 Signal	Corrected S1 Signal	Corrected S2 Signal	Sample Specific S1 Ratio (Sample Corrected S1 Signal / Average S1 Signal High Calibrator)	Sample Specific S2 Ratio (Sample Corrected S2 Signal / Average S2 Signal High Calibrator)
High Calibrator						
Sample 1						
Sample 2						

Step 4: Determine the presence of reactive SARS-CoV-2 Antibodies

	Data Input				Calculations		
	S1 Cutoff Value	S2 Cutoff Value	Sample Specific S1 Ratio	Sample Specific S2 Ratio	S1 Positive (Sample Specific S1 Ratio > S1 Cutoff Value)	S2 Positive (Sample Specific S2 Ratio > S2 Cutoff Value)	SARS-CoV-2 Reactive Antibodies Present (Both S1 and S2 are Positive)
Cutoff							
Sample 1							
Sample 2							

ABBREVIATED PROTOCOL

Preparation

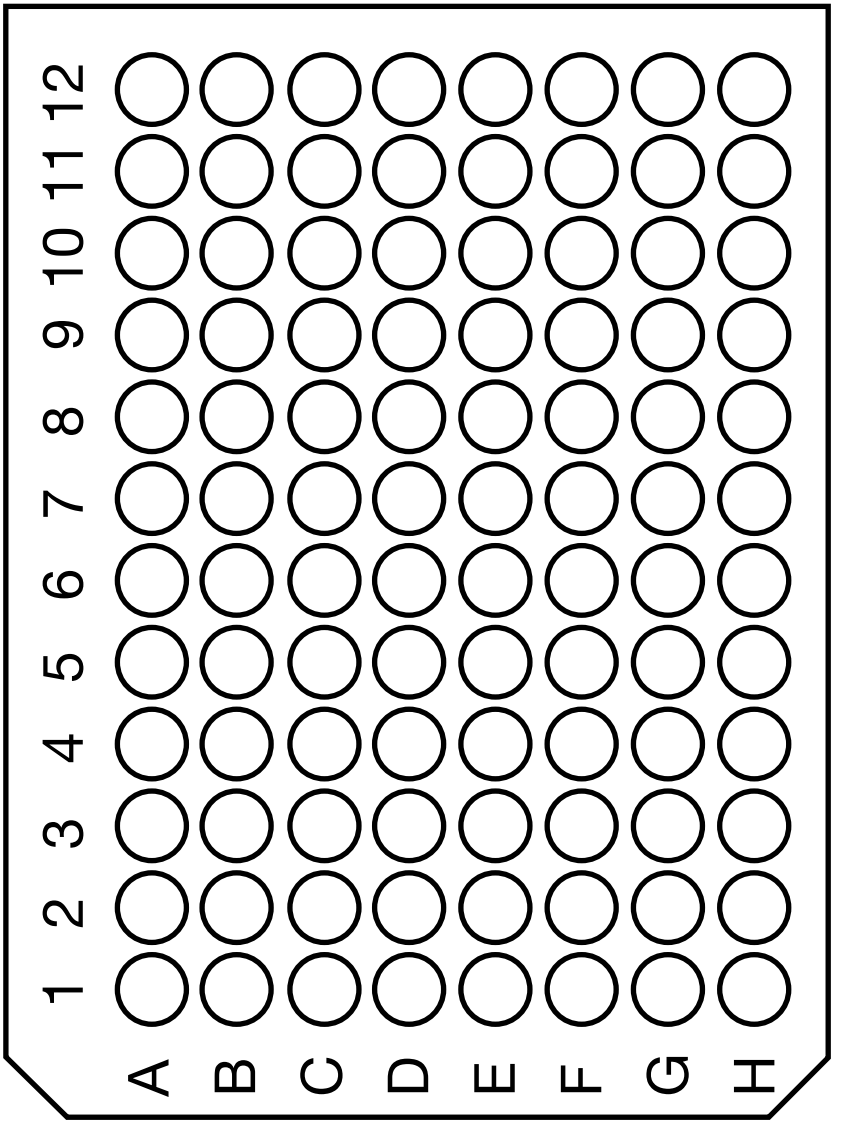
1. Install Q-View Software *(page 11)*.
2. Set up imager *(page 11)*.
3. Set up microplate washer *(page 27)*.
4. Reconstitute and prepare reagents *(page 11).*

Running the Assay

5. Prepare the calibrators by reconstituting with Sample Diluent according to the Product Card *(page 11)*.
6. Prepare the samples with Sample Diluent *(page 12)*.
7. Load the calibrators and samples onto the plate. Incubate for one hour at room temperature *(page 12)*.
8. Wash the plate three times, add the Detection Mix, and incubate for thirty minutes at room temperature *(page 12)*.
9. Wash the plate three times, add the Streptavidin-HRP 1X, incubate for twenty minutes at room temperature *(page 12)*.
10. Allow Substrate A and Substrate B+ to come to room temperature, then mix equal volumes and allow the solution to sit at room temperature *(page 11)*.
11. Wash the plate six times and add the mixed Substrate *(page 12)*.
12. Capture and analyze image of the plate *(page 12)*.

We take great care to ensure that customers have success using our products and services. If you have further questions about running the assay, data analysis, troubleshooting, or our products or services, please contact us at 888-QUANSYS (782-6797) or at support@quansysbio.com.

PLATE DIAGRAM



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