QUANSYS BIOSCIENCES

Q-Plex™ ARRAY

SARS-CoV-2 Human IgG Quantitative (4-Plex)

For Research Use Only Version 1.1
Not For Use In Diagnostic Procedures

TABLE OF CONTENTS

Symbol	Explanation
REF	Catalog Number
LOT	Lot Number
	Use By YYYY-MM-DD
	Temperature Limitation
	Manufacturer
	Keep Away From Sunlight

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NAME AND INTENDED USE

Q-Plex™ SARS-CoV-2 Human IgG Quantitative (4-Plex)
Quansys Biosciences Catalog Number 711649HU

The Q-Plex SARS-CoV-2 Human IgG Quantitative (4-Plex) assay is a quantitative 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. The intended use of the kit is for research use only.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the microplate indirect enzyme immunoassay technique for the measurement of human IgGs reactive to SARS-CoV-2 S1, SARS-CoV-2 S2, Sheep Fc (negative control), and total human IgG (positive control).

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) 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 detect cross-reactivity that may be present 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 assay protocol was followed correctly.

Samples, calibrator, or 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 IgG, a mixture that contains biotinylated anti-human IgG is added. 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 human IgG antibody reactive to SARS-CoV-2 S1, SARS-CoV-2 S2, Sheep Fc (negative control), Anti-human IgG (positive control) 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. 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. Diluted samples 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 EDTA, Citrate, or 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.

Fingerstick – VAMS blood samples are collected using the Mitra® 10µL collection samplers (Neoteryx, CA, USA). Collect a droplet of blood from a fingerstick onto the porous, hydrophilic VAMS tip until completely filled with blood. Each tip absorbs 10 µL of blood, and two tips are present in each collection kit, for a total of 20 µL of blood per collection. Place the cassette containing the two tips in a biohazard specimen bag containing a desiccant pack. The samples can be stored for up to 2 weeks before testing.

WARNINGS AND PRECAUTIONS

1. Read all instructions before beginning test.
2. The kit should not be used beyond the expiration date on the kit label.
3. If running multiple kits, a calibration curve must be included for each 96-well plate. The control values from one plate cannot be used to determine sample results from other plates.
4. Do not mix or substitute reagents with those from other kits or lots.
5. 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 or controls to the microplate.
6. Load all calibrator, controls, and samples into the microplate within 5 minutes of each other.
7. Be exact with incubation times, particularly the streptavidin-horseradish peroxidase (SHRP) incubation.
8. Be exact when mixing Substrate A and B+ and mix thoroughly.
9. Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade them.
10. Do not allow the plate to dry out between steps.
11. Warning: The controls and calibrator 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	
Calibrator Lyophilized, human serum diluted in a buffered protein base	 2-8°C until kit expiration Discard unused reconstituted calibrator.
Controls (High and Low) Lyophilized, human serum diluted in a buffered protein base	 2-8°C until kit expiration Discard unused reconstituted controls.
Streptavidin-HRP 1X Liquid, 6 mL/vial of streptavidin-conjugated horseradish 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 (20-1000 µL) and/or single channel pipettes (2-1000 µL) and tips
2. 10 mL serological pipette
3. Polypropylene tubes or polypropylene 96-well plate(s) for sample and calibrator preparation
    a. Example: Nunc® MicroWell™ 96-Well Plates, Polypropylene, 249944; Eppendorf® LoBind Protein or Genomic Microcentrifuge Tubes, 022431102
4. Q-View™ Imager and Software
5. Deionized water
6. Microplate washer (An optional multichannel pipette protocol is provided in Appendix A.)
7. One liter graduated cylinder for the preparation of wash buffer
8. 50 mL conical tube or other container for diluting the 2x Sample Diluent

ASSAY PREPARATION

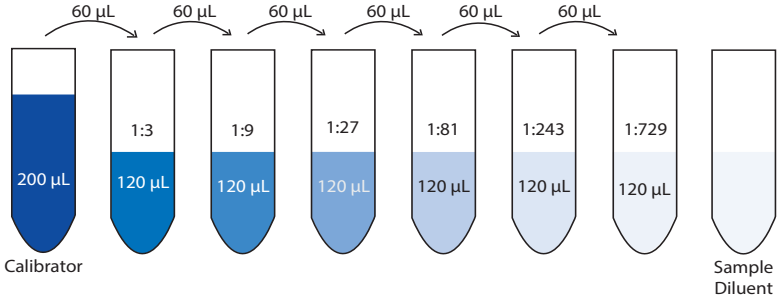
1. Install the Q-View™ Software on the computers that will be used for analysis or operating a Q-View™ Imager Pro or Q-View™ Imager LS.
2. Set up the imager. For imager-specific instructions, see www.quansysbio.com/manuals.
3. Prepare Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized 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 Calibrator and Controls: Reconstitute using 1X Sample Diluent in accordance with the volume specified on the Product Card which accompanies the kit. Allow Controls and Calibrator 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 that all calibrators 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 µL of prepared calibrator into the first tube or well.
    b. Place 120 µL of prepared sample diluent into the other seven tubes or wells.

c. Transfer 60 µL 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 below. The undiluted prepared calibrator serves as the high point of the standard curve. The prepared sample diluent serves as the negative.



2. **Serum and Plasma Samples:** Prepare samples by diluting 1:100 (one part sample to ninety-nine parts prepared sample diluent) with enough 1X Sample Diluent to have 50 µL of diluted sample per well in either polypropylene tubes or a polypropylene 96-well plate.

3. **Fingerstick samples on Mitra 10 µL sampler:** Reconstitute the sampler in 120 µL of sample diluent for 30 minutes with shaking. Dilute the sample eluate one part eluate into three parts sample diluent with enough volume to have 50 µL of diluted sample per well.

4. Add 50 µL per well of the calibration curve and controls to duplicate wells of the microarrayed plate.

5. Add 50 µL per well of the diluted samples to either single or duplicate wells. Load all calibrators, samples, and controls to the plate within five minutes.

6. Cover the plate with a plate seal provided and **incubate for one hour** at room temperature (20-25°C).

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

8. 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).

9. Wash the plate three times (see Appendix A).
10. 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).
11. Wash the plate six times (see Appendix A).
12. Add 50µL 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 5 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.
13. Place the plate in the Q-View Imager Pro or Q-View Imager LS.
14. Open Q-View Software, create or open a project, and click Acquire Image.
15. When using a Q-View Imager Pro, set the exposure time to 300 seconds.
16. When using a Q-View Imager LS, set the exposure time to 270 seconds.
17. Click the Capture Image(s) button. Users may continue 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**.
18. 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 of the software.
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** 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 or controls. Use **Templates** to quickly assign layouts that are repeated often or export the layout as a .csv file.
5. **Data Analysis:** Once you have completed **Image Processing** and **Well Assignment**, select **Data Analysis**. Click **Perform Analysis** to generate charts and statistics.

6. **Interpretation of Results:** The assay uses a calculated cutoff value (explained below) to establish the ranges used to determine positive and negative results.

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

      - 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. Samples with an error code AC4 are invalid.
      - The Sheep Fc spot is a negative control to detect cross-reactivity that may be present between human IgGs in the sample and the Fc-Tag on the SARS-CoV-2 Spike proteins. Q-View software will report an error code (AC3) in the Report tab for any sample that produces a signal that indicates there is cross-reactivity. Samples with error code AC3 are invalid.
      - 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.
      - High and Low Control Samples must meet their expected ranges. These ranges can be found on the Certificate of Analysis. Q-View Software will report an error code if controls are not in their expected ranges.
      - The user should review any error codes reported by Q-View software.

b. **Determine if samples are positive on S1 and S2:** Compare the sample concentration to the cutoff for each assay found on the certificate of analysis. Samples with a higher concentration than the S1 or S2 cutoff are positive for antibodies to the S1 or S2 protein.

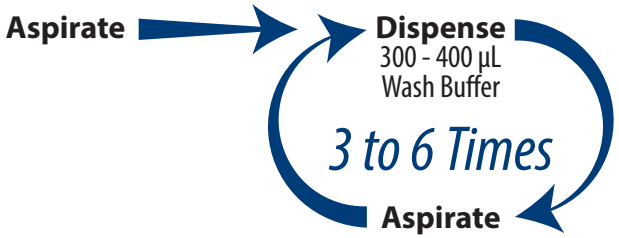
c. **Only samples that are positive on both the S1 and S2 assay are considered to have reactive antibodies to SARS-CoV-2.**

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.

APPENDIX A: PLATE WASHING METHOD

Automated Wash Method

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 and 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.

ABBREVIATED PROTOCOL

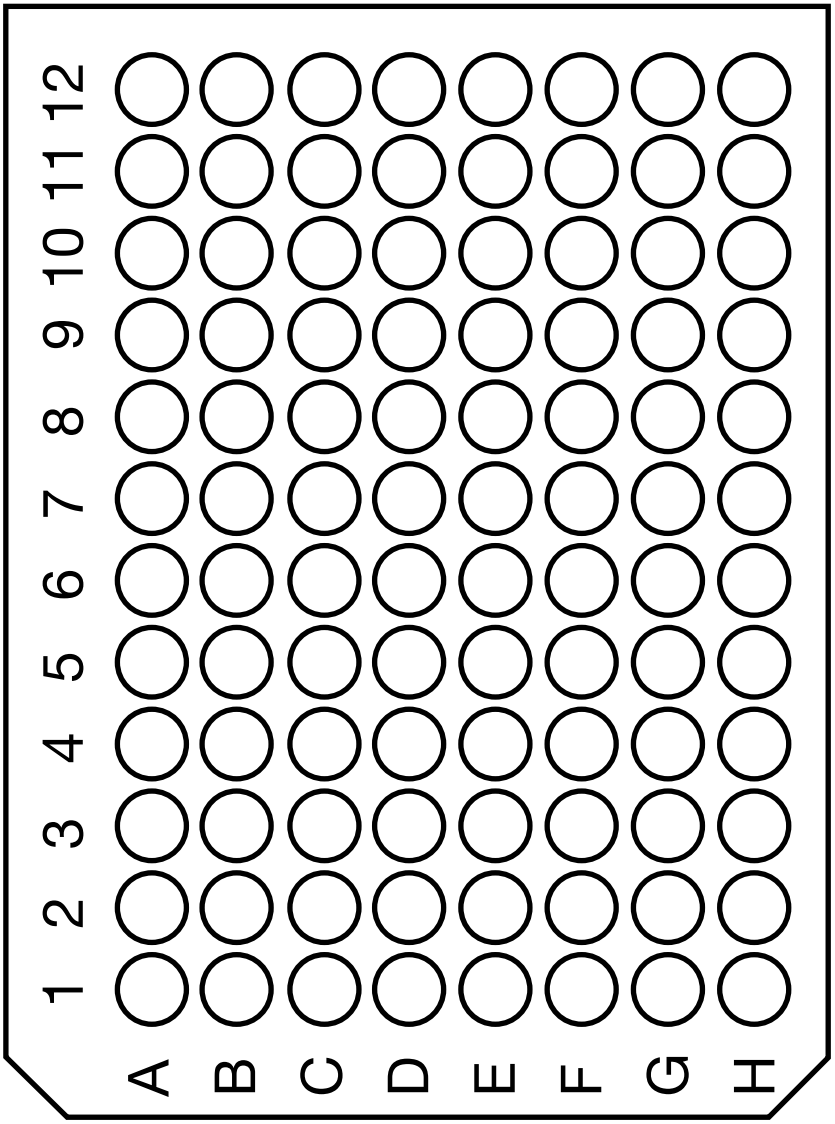
Preparation

1. Install Q-View Software *(page 8)*.
2. Set up the imager *(page 8)*.
3. Set up microplate washer *(page 14)*.
4. Reconstitute and prepare reagents *(page 8).*

Running the Assay

5. Prepare the calibration curve and controls using the Calibrator and Controls and reconstituting with Sample Diluent according to the Product Card *(page 8)*.
6. Prepare the samples with Sample Diluent *(page 9)*.
7. Load the calibrators, controls, and samples in the plate. Incubate for one hour at room temperature *(page 9)*.
8. Wash the plate three times, add the Detection Mix, and incubate for thirty minutes at room temperature *(page 9)*.
9. Wash the plate three times, add the Streptavidin HRP 1X, incubate for twenty minutes at room temperature *(page 10)*.
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 8)*.
11. Wash the plate six times and add the mixed Substrate *(page 10)*.
12. Capture and analyze image of the plate *(page 10)*.

PLATE DIAGRAM



NOTES

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