QUANSYS BIOSCIENCES

Q-Plex™ Human Micronutrient v2 (7-Plex)

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

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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™ Human Micronutrient v2 (7-Plex)
Quansys Biosciences Catalog Number 355149HU

The Q-Plex™ Human Micronutrient v2 (7-Plex) is a quantitative chemiluminescent assay (ELISA) allowing concurrent measurement in serum or heparin plasma samples of α-1-acid glycoprotein (AGP), C-reactive protein (CRP), ferritin, histidine-rich protein II (HRP2), retinol binding protein 4 (RBP4), soluble transferrin receptor (sTfR), and thyroglobulin biomarkers (Tg). This kit is intended for research use only.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the sandwich enzyme immunoassay technique for the measurement of CRP, Ferritin, HRP2, sTfR and Tg and the competitive enzyme immunoassay technique for the measurement of AGP and RBP4.

The sandwich assays use 2 different antibodies specific for their respective targets. Samples or calibrators are pipetted into wells of a 96 well plate arrayed with immobilized analyte-specific antibodies that capture CRP, ferritin, HRP2, sTfR, and Tg. After washing away any unbound protein, 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 bound to each location of the array is proportional to the amount of CRP, ferritin, HRP2, sTfR, and Tg initially captured.

The AGP and RBP4 assays use an antibody specific for their respective targets. Samples or calibrators are pipetted into wells of a microplate arrayed with immobilized analyte-specific antibodies that capture AGP and RBP4. During the sample incubation, AGP and RBP4 present in a sample competes with a fixed amount of biotin-labeled AGP and RBP4 for sites on the immobilized antibody. This is followed by a wash to remove excess biotin-labeled AGP and RBP4 and unbound protein. At this point of the multiplex protocol, the competitive assays act as spectators during the biotinylated antibody incubation step of the sandwich assays. After washing away unbound biotinylated antibody, SHRP is added. Following an additional wash, the amount of SHRP remaining on each location of the array is inversely proportional to the amount of AGP and RBP4 initially present in a sample.

The amount of conjugated enzyme on each location of the array is measured with the addition of a chemiluminescent substrate.

Assay Ranges

Please refer to accompanying In-Kit Certificate of Analysis.

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.

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.

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.

WARNINGS AND PRECAUTIONS

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.
4. 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.
5. Do not mix or substitute reagents with those from other kits or lots.
6. Pre-wet pipette tips 3 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.
7. Load all calibrators and samples into the microplate within 10 minutes of each other.
8. Be exact with incubation times, particularly the streptavidin-horseradish peroxidase (SHRP) incubation.
9. Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade it.
10. Do not allow the plate to dry out between steps.
11. Be exact when mixing Substrate A and B2 and mix thoroughly.
12. 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.

KIT CONTENTS, PREPARATION, & 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 (355152HU) Arrayed and blocked 96-well polystyrene microtiter plate	 2-8°C until kit expiration
Wash Buffer Concentrate (20X) (101158GR) Liquid, 50 mL/vial of a concentrated solution of buffered surfactant	
Sample Diluent 2X (355157HU) Liquid, 10 mL/vial of a buffered protein solution with heterophilic antibody and rheumatoid factor blockers and preservatives	
Competitor (355190HU) Lyophilized, biotinylated competitive antigen	
Detection Mix (355156HU) Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives	
Lyophilized Calibrator (355155HU) Lyophilized, recombinant or purified antigens in a buffered protein base	 2-8°C until kit expiration Discard unused reconstituted calibrator.
Calibrator Additive (355142HU) Purified ferritin antigen in a buffered protein base	 2-8°C until kit expiration
Streptavidin-HRP (101173GR) Liquid, 6 mL/vial of streptavidin-conjugated horseradish peroxidase	 Do not expose to UV light.  2-8°C until kit expiration
Substrate A (101159GR) Liquid, 3.5 mL/vial	 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 B2 (101128GR) Liquid, 3.5 mL/vial	
Plate Seals (3) (S1064) 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 (20-100 µL) and/or single channel pipettes (20-1000 µL) and tips
2. 10 mL serological pipette
3. 1 liter graduated cylinder for the preparation of wash buffer
4. Polypropylene tubes or polypropylene 96-well plate(s) for sample and calibrator preparation
    a. For Example: Nunc® MicroWell™ 96-Well Plates, Polypropylene, 249944; Eppendorf® Protein LoBind Microcentrifuge Tubes, 022431102
5. Microplate washer (An optional multichannel pipette protocol is provided in Appendix A)
6. Deionized water
7. Q-View™ Imager and Q-View™ Software

ASSAY PREPARATION

1. Install Q-View Software on the computer that will be used for operating a Q-View Imager.
2. Set up the imager. For imager-specific instructions, see www.quansysbio.com/manuals.
3. Allow all kit components to come to room temperature (20-25°C) prior to use.
4. Prepare Wash Buffer: Place 50 mL of Wash Buffer Concentrate (20X) into 950 mL deionized water and mix thoroughly.
5. Prepare 1X Sample Diluent: Dilute Sample Diluent 2X with 10 mL of deionized water.
6. Prepare Complete Sample Diluent: Add 1 mL of 1X Sample Diluent to lyophilized Competitor. Allow to sit for 5 minutes. Mix thoroughly. Return the 1 mL back into the remaining 19 mL of 1X Sample Diluent. Mix thoroughly.
7. Prepare Calibrator:
   - Step 1: Reconstitute Lyophilized Calibrator using Complete Sample Diluent with the volume specified on the In-Kit Certificate of Analysis accompanying the kit.
   - Step 2: To the reconstituted Lyophilized Calibrator, add the volume of Calibrator Additive specified on the In-Kit Certificate of Analysis. Allow to sit for 5 minutes. Mix thoroughly.

   Use prepared calibrator within 30 minutes.
8. Prepare chemiluminescent substrate: Combine 3 mL of Substrate A with 3 mL of Substrate B2 and mix gently. Do not expose to UV light. Store at room temperature (20-25°C) after mixing.

ASSAY PROCEDURE

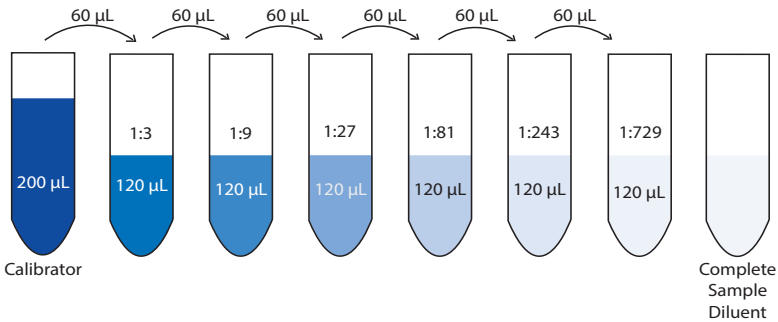
Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed by the previous sections. It is recommended that all calibrators, samples, and controls be assayed in duplicate.

1. Using the previously prepared 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 complete sample diluent into the other 7 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 complete sample diluent serves as a blank.



2. Dilute samples 1:40 (1 part sample to 39 parts complete 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. A recommended dilution is 5 µL sample diluted into 195 µL complete sample diluent.

Note: If you anticipate that your analyte concentrations will be greater than the high point of the calibration curve as reported on the In-Kit Certificate of Analysis, use the complete sample diluent to dilute your samples further.

3. Add 50 µL per well of the calibration curve to duplicate wells of the Q-Plex Array Microplate.
4. Add 50 µL per well of diluted samples to either single or duplicate wells. Load all samples and calibration curve to the plate within 10 minutes.
5. Cover the plate with a provided plate seal and incubate for 120 minutes at room temperature (20-25°C).
6. Wash the plate 3 times (see Appendix A).
7. Add 50 µL per well of Detection Mix, cover with a new plate seal, and incubate for 60 minutes at room temperature (20-25°C).
8. Wash the plate 3 times (see Appendix A).
9. Add 50 µL per well of Streptavidin-HRP, cover with a new plate seal, and incubate for 20 minutes at room temperature (20-25°C).
10. Wash the plate 6 times (see Appendix A).
11. Add 50 µL per well of previously prepared chemiluminescent 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.
12. Place the plate in the Q-View Imager.
13. Open Q-View Software, create or open a project, and click Acquire Image.
14. When using a Q-View Imager Pro, set the exposure time to 300 seconds.
15. When using a Q-View Imager LS, set the exposure time to 270 seconds and standard image processing.
16. Click the Capture Image(s) button. Users may perform 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**.

17. Dispose of all used and unused materials in accordance with local regulations.

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**.

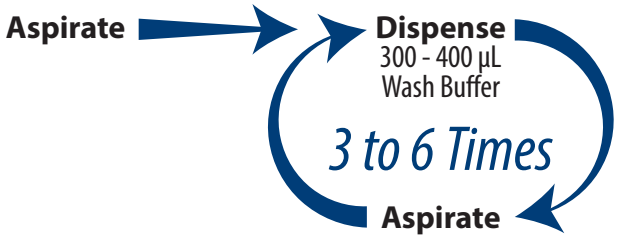
1. Acquire or import an image into Q-View Software as previously described.
2. Enter the Software **Product Code** (found on the In-Kit Certificate of Analysis) into the **Product Code** field of the software.
3. **Image Processing**: Align the plate overlay as follows:
    a. Set the overlay: If using 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 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, controls, calibrators, or negatives and specify their dilution factors. Use **Templates** to quickly assign layouts that are commonly repeated 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, concentrations, and statistics based on optimal regression settings provided by the product definition.
6. Samples above the ULOQ of the assay should be retested at a higher dilution. A dilution of 1:200 is recommended but others may be used as needed.

We take great care to ensure that customers can successfully use 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 METHODS

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 and 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 1 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 1 time. When the assay procedure calls for 3 or 6 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

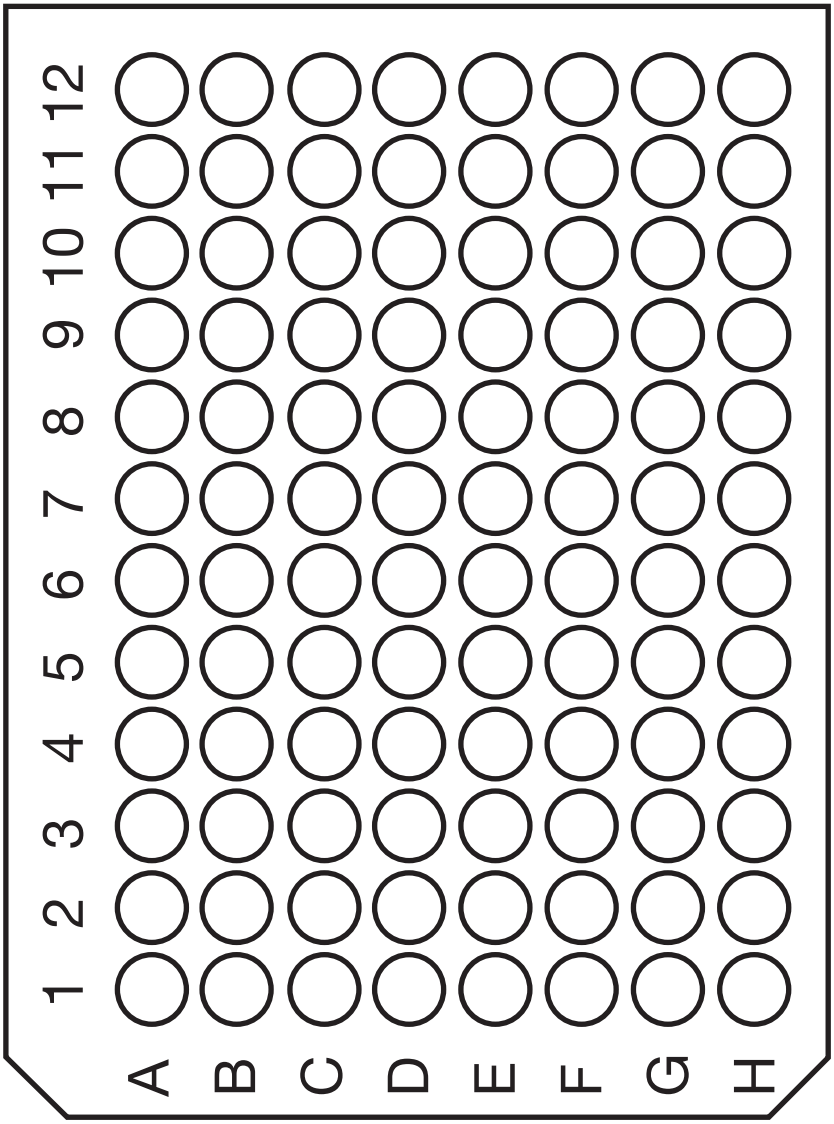
Preparation

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 using the Prepared Calibrator and Complete Sample Diluent according to the In-Kit Certificate of Analysis (page 9).
6. Prepare the samples with Complete Sample Diluent (page 9).
7. Load the calibration curve and samples onto the plate. Incubate for 120 minutes at room temperature (page 10).
8. Wash the plate 3 times, add the Detection Mix, and incubate for 60 minutes at room temperature (page 10).
9. Wash the plate 3 times, add the Streptavidin-HRP, incubate for 20 minutes at room temperature, (page 10).
10. Wash the plate 6 times and add the mixed Substrate (page 10).
11. Capture and analyze image of the plate (page 10).

PLATE DIAGRAM



NOTES

Q-Plex™ ARRAY

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355117HU

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