QUANSYS BIOSCIENCES

Q-Plex™ Human Environmental Enteric Dysfunction (11-Plex)

For Research Use Only Version 1.2
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™ Human Environmental Enteric Dysfunction (11-Plex)
Quansys Biosciences Catalog Number 569849HU

The Q-Plex Human Environmental Enteric Dysfunction (11-Plex) is a quantitative chemiluminescent ELISA allowing concurrent measurement in serum and heparin plasma samples of IGF-1, FGF-21, IFABP, Ferritin, HRP2, sTfR, Thyroglobulin, AGP, CRP, CD-14 and RBP4 biomarkers.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the microplate sandwich enzyme immunoassay technique for the measurement of IGF-1, FGF-21, IFABP, Ferritin, HRP2, sTfR and Thyroglobulin and the microplate competitive enzyme immunoassay technique for the measurement of AGP, CRP, CD-14 and RBP4.

The IGF-1, FGF-21, IFABP, Ferritin, HRP2, sTfR and Thyroglobulin assays use two different antibodies specific for their respective targets. Samples or calibrators are pipetted into wells of a microplate arrayed with analyte specific antibodies that capture IGF-1, FGF-21, IFABP, Ferritin, HRP2, sTfR and Thyroglobulin, thereby immobilizing IGF-1, FGF-21, IFABP, Ferritin, HRP2, sTfR and Thyroglobulin to their locations in the array. 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 remaining on each location of the array is proportional to the amount of IGF-1, FGF-21, IFABP, Ferritin, HRP2, sTfR and Thyroglobulin initially captured.

The AGP, CRP, CD-14 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, CRP, CD-14 and RBP4. During the sample incubation, AGP, CRP, CD-14 and RBP4 present in a sample competes with a fixed amount of biotin-labeled AGP, CRP, CD-14 and RBP4 for sites on the immobilized antibody. This is followed by a wash to remove excess biotin-labeled AGP, CRP, CD-14 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, CRP, CD-14 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. Sample stability has not been evaluated.

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.

IMPORTANT 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. 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.
7. 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.

DO

- Do set up and practice using the Q-View™ Imager Pro or Q-View™ Imager LS before starting the assay.
- Do be exact when setting shaker speed to 500 RPM.
- Do dilute all samples at least 1:10 (one part sample to nine parts diluent) with the provided sample diluent to prevent false positives, and mix thoroughly.
- Do 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 calibrators to the microplate.
- Do load all calibrators and samples into the microplate within five minutes of each other.
- Do be exact with incubation times, particularly the streptavidin-horseradish peroxidase (SHRP) incubation.
- Do be exact when mixing Substrate A and B2 and mix thoroughly.

DO NOT

- 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.

KIT CONTENTS, PREPARATION, & STORAGE

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

Part/Description	Storage of opened/reconstituted material
Q-Plex™ Array Microplate 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 Liquid, 10 mL/vial of a buffered protein solution with heterophilic antibody and rheumatoid factor blockers and preservatives	
Competitor Lyophilized, biotinylated competitive antigen	
Detection Mix Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives	
Calibrator Lyophilized, recombinant antigens in a buffered protein base	 Store at -20°C until use Discard unused reconstituted calibrator.
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 stabilized hydrogen peroxide	 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 Liquid, 3 mL/vial of stabilized signal enhancer	
Plate Seals (3) Adhesive strips	Non-perishable

OTHER REQUIRED MATERIALS: INSTRUMENTS AND ACCESSORIES

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) and tips
2. Polypropylene tubes or polypropylene 96-well plate(s) for sample and calibrator preparation
    a. Recommended: Nunc® MicroWell™ 96-Well Plates, Polypropylene, 249944; Eppendorf® LoBind Protein or Genomic Microcentrifuge Tubes, 022431102
3. Q-View™ Imager and Software
4. Microplate shaker
    a. For example: Barnstead/labline 4625 titer plate shaker, IKA MTS 2/4 for 2 or 4 microtiter plates, or equivalent, capable of 500 RPM.
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

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. Set the plate shaker to 500 RPM.
4. Prepare the Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water, and mix thoroughly.
5. Prepare Sample Diluent: Add 1 mL of Sample Diluent to Competitor Mix. Allow Competitor to sit for 5 minutes. Mix thoroughly. Return the 1 mL back into the remaining 9 mL vial of Sample Diluent. Mix thoroughly.
6. Prepare Calibrator: Reconstitute using Sample Diluent containing competitor with the volume on the In-Kit Certificate of Analysis which accompanies the kit. Allow Calibrator to sit for 5 minutes. Mix thoroughly. Use calibrator within 30 minutes of reconstitution.
7. Allow Substrate A and B2 to come to room temperature (20-25°C). Fifteen minutes prior to use, 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.**

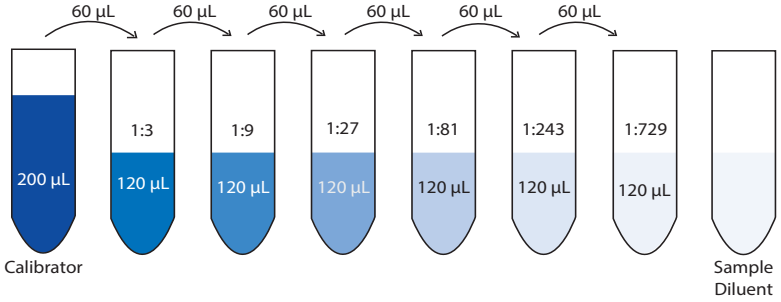
ASSAY PROCEDURE

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 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. Prepare plasma samples by diluting 1:10 (one part sample to nine parts prepared sample diluent) with enough prepared sample diluent to have 50 µL 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 curve as reported on the In-Kit Certificate of Analysis included in the kit, use the prepared sample diluent to dilute your samples further.

3. Add 50 µL 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 µL and dispensing into the Q-Plex™ Array 96-well plate. Load all samples and calibration curve to the plate within five minutes.

4. Cover the plate with a plate seal provided, and shake on a plate shaker set to 500 RPM for two hours 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 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 µL per well of Streptavidin-HRP 1X, cover with a new plate seal, and return to the plate shaker set to 500 RPM for 20 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. 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. When using a Q-View Imager LS, set the exposure time to 270 seconds and standard image processing.
14. 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**.
15. Dispose of all used and unused materials. Disposal of waste may differ from region to region. 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**.

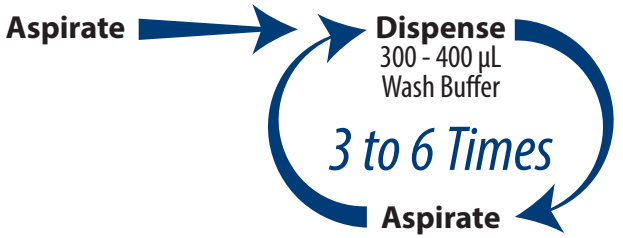
1. Acquire or import an image into Q-View Software as previously described.
2. Enter the **Software Product Code** (found on the In-Kit Certificate of Analysis) into the **Product Code** field of the software.
3. **Image Processing**: Align the plate overlay as follows:
    a. Set the overlay: If using 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 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, concentrations, and statistics based on optimal regression settings provided by the product definition.

    Note: Tips for data analysis are available at www.quansysbio.com/tech-tips. 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 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	3.810 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. To ensure that all pins are functioning, 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.
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

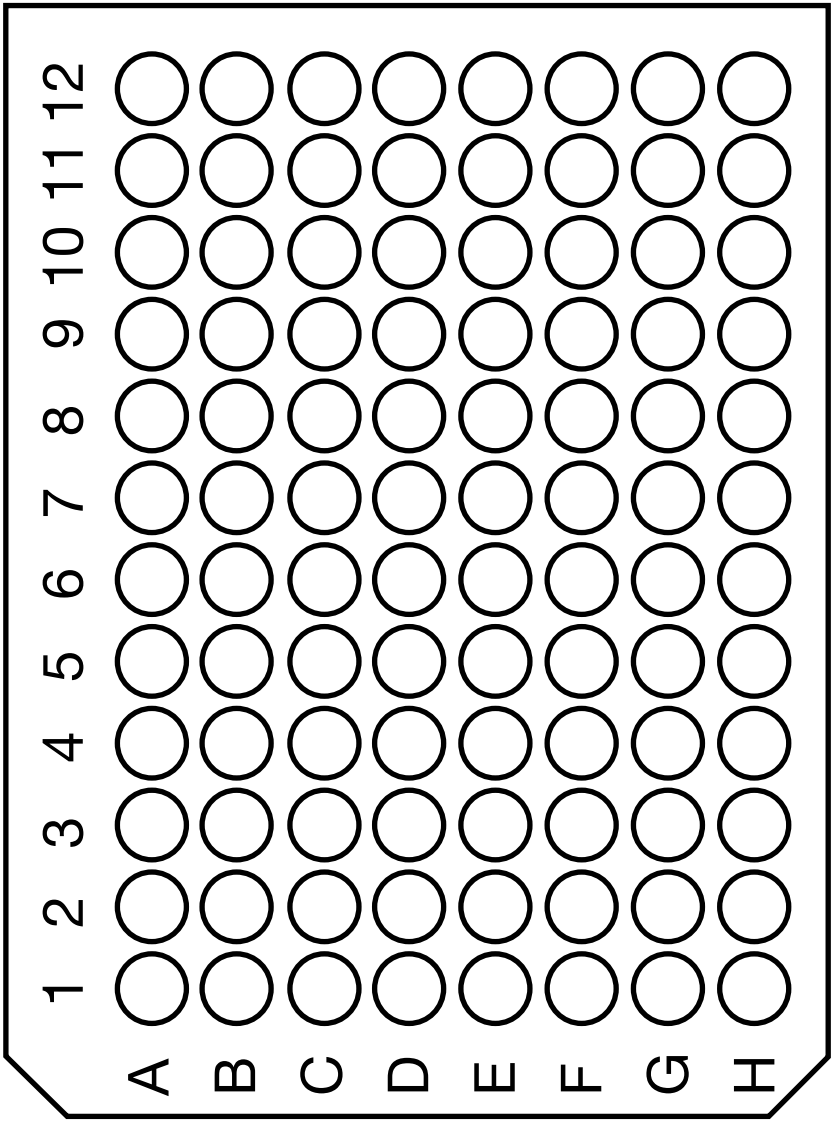
Preparation

1. Install Q-View Software *(page 8)*.
2. Set up the imager *(page 8)*.
3. Set up microplate washer *(page 12)* and shaker *(page 8).*
4. Reconstitute and prepare reagents *(page 8).*

Running the Assay

5. Prepare the calibration curve using the Calibrator and Sample Diluent containing competitor according to the In-Kit Certificate of Analysis *(page 8)*.
6. Prepare the samples with Sample Diluent containing competitor *(page 9)*.
7. Load the calibration curve and samples onto the plate. Shake for two hours at room temperature (500 RPM) *(page 9)*.
8. Wash the plate three times, add the Detection Mix, and shake for one hour at room temperature (500 RPM) *(page 9)*.
9. Wash the plate three times, add the Streptavidin HRP 1X, shake for 20 minutes at room temperature (500 RPM) *(page 10)*.
10. Allow Substrate A and Substrate B2 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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