QUANSYS BIOSCIENCES

Q-Plex™ ARRAY

Rat Cytokine Inflammation (9-Plex)

For Research Use Only Version 3.2
Not For Use In Diagnostic Procedures

QUANSYS BIOSCIENCES

TABLE OF CONTENTS

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


Manufactured and Distributed by:
Quansys Biosciences
365 North 600 West Logan, Utah 84321, USA
TEL: (888) 782-6797
E-MAIL: INFO@QUANSYSBIO.COM

For International Distributors see:
http://www.quansysbio.com/distributors

NAME AND INTENDED USE

Q-Plex™ Rat Cytokine Inflammation (9-Plex)
Quansys Biosciences Catalog Number 111649RT

The Q-Plex Rat Cytokine Inflammation (9-plex) is a quantitative chemiluminescent assay (ELISA) allowing concurrent measurement in rat serum, EDTA plasma, and cell culture supernatant samples of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL- 12p70, IFN-γ, and TNF-α biomarkers. This kit is intended for research use only.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the 96 well plate sandwich enzyme immunoassay technique for the measurement of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL- 12p70, IFN-γ, and TNF-α.

These 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 IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL- 12p70, IFN-γ, and TNF-α. 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 IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IFN-γ, and TNF-α initially captured.

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 is 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 before testing. Diluted samples should be tested within 8 hours. Do not use bacterially contaminated samples. It is the individual laboratory's responsibility to use all available references and/or its own studies to determine its own specific stability criteria.

Cell Culture Supernatant - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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

Plasma - Collect plasma using EDTA 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.

WARNINGS AND PRECAUTIONS

1. Read all instructions before beginning the 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 by drawing up the liquid into the pipette and then dispensing back into the original vessel before adding samples or controls to the microplate.
7. Load all calibrators and samples into the microplate within 20 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 B+ and mix thoroughly.
12. Be exact when setting shaker speed to 500 RPM.

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 blockers and preservatives	
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	Discard unused reconstituted calibrator.
Streptavidin-HRP 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	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	
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 (20-1000 µL) and tips
2. 10 mL serological pipette
3. 1 L 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® LoBindProtein or Genomic Microcentrifuge Tubes, 022431102
5. Q-View™ Imager and Software
6. 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
7. Microplate washer (An optional multichannel pipette protocol is provided in Appendix A)
8. Deionized water

ASSAY PREPARATION

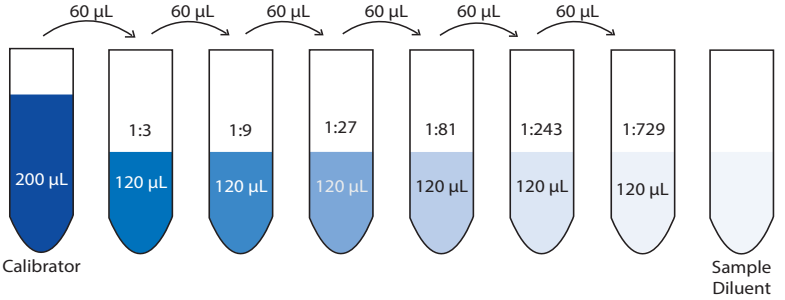
1. Install Q-View™ Software on the computer that will be used for analysis or operating a Q-View™ Imager Pro or Q-View™ Imager LS.
2. Set up the imager. For imager-specific instructions, see www.quansysbio.com/manuals.
3. Set the plate shaker to 500 RPM.
4. Prepare wash buffer: Place 50 mL of 20X concentrate into 950 mL deionized water and mix thoroughly.
5. Prepare calibrator: Reconstitute using sample diluent with the volume specified on the In-Kit Certificate of Analysis accompanying the kit. Allow the calibrator to sit for 5 minutes. Mix thoroughly. Use the calibrator within 30 minutes of reconstitution.
6. Allow Substrate A and B+ to come to room temperature (20-25°C). 15 minutes before 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 samples 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 7 tubes or wells.

c. Transfer 60 µL of 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. Dilute samples 1:2 (1 part sample to 1 part 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 of the microarrayed plate.

   Note: If you anticipate that your analyte concentrations will be greater than the high point of the calibration curves 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 to duplicate wells of the microarrayed plate.

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 20 minutes.

5. Cover the plate with a provided plate seal and shake on a plate shaker set to 500 RPM for 90 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 return to the plate shaker set to 500 RPM 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 return to the plate shaker set to 500 RPM 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 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 before 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 Pro or Q-View Imager LS.
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. 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 Imager and Software Manuals, viewable at www.quansysbio.com/manuals, or within Q-View Software under **Support > Manual**.

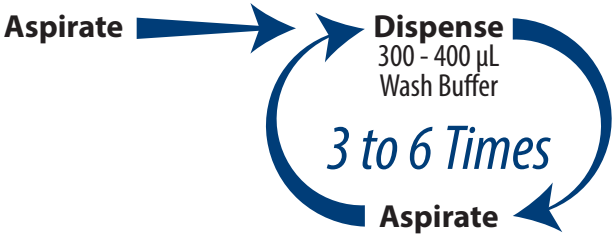
1. Acquire or import an image into Q-View Software as previously described.
2. Enter the **Software Product Code** (found on the In-Kit Certificate of Analysis) into the software's **Product Code** field.
3. **Image Processing**: Align the plate overlay as follows:
    a. Set the overlay: If using the **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, controls, calibrators or negatives, and specify their dilution factors. Use **Templates** to quickly assign layouts that are commonly repeated or to 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.

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 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	3.810 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 ensure that 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 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 before 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. Repeat steps 3 and 4 for a total of 3 or 6 washes in accordance with the assay protocol.
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

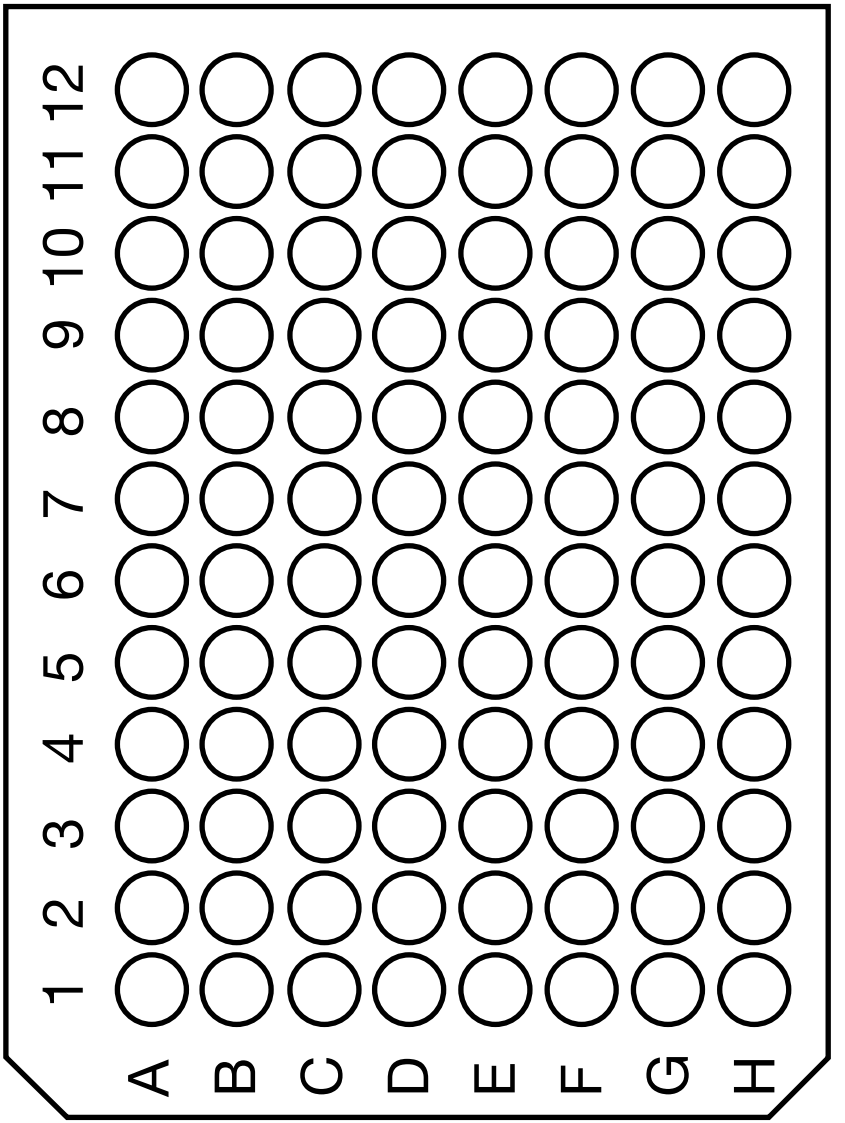
Preparation

1. Install Q-View Software *(page 7)*.
2. Set up the imager *(page 7)*.
3. Set up microplate washer *(page 11)* and shaker *(page 7).*
4. Reconstitute and prepare reagents *(page 7).*

Running the Assay

5. Prepare the calibration curve using the calibrator and sample diluent according to the In-Kit Certificate of Analysis (page 7).
6. Prepare the samples with sample diluent (page 8).
7. Load the calibration curve and samples onto the plate. Shake for 90 minutes at room temperature (500 RPM) (page 8)
8. Wash the plate 3 times, add Detection Mix and shake for 60 minutes at room temperature (500 RPM) (page 8).
9. Wash the plate 3 times, add Streptavidin HRP and shake for 20 minutes at room temperature (500 RPM) (page 9).
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 7).
11. Wash the plate 6 times and add the mixed substrate (page 9).
12. Capture and analyze the image of the plate (page 9).

PLATE DIAGRAM



www.quansysbio.com

365 North 600 West, Logan, Utah 84321

T: 1-888-782-6797 • F: (435)750-6869

www.quansysbio.com • Technical Support: support@quansysbio.com

111617RT