QUANSYS BIOSCIENCES

Q-Plex™ Bovine Cytokine Panel 1 (5-Plex)

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

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

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QUANSYS BIOSCIENCES

NAME AND INTENDED USE

Q-Plex™ Bovine Cytokine Panel 1 (5-Plex)

Quansys Biosciences Catalog Number 112349BV

The Q-Plex Bovine Cytokine Panel 1 (5-Plex) is a chemiluminescent Enzyme-Linked Immunosorbent Assay (ELISA) intended for quantitative concurrent measurement of cytokines IFNγ, IL-4, IL-10, IL-12 p40, and TNFα, in serum, plasma, and cell supernatant samples.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the microplate sandwich enzyme immunoassay technique for the measurement of cytokines.

Sandwich Assays use two 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 antigens. 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 antigen 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. 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. This assay has shown sensitivity to hemoglobin levels above 1.25 mg/mL. It is not recommended to run samples containing interferent at levels higher than specified due to interference with assay performance on at least one analyte.

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, 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.

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, calibrators, or external controls to the microplate.
7. Load all calibrators, controls, 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. Be exact when mixing Substrate A and B+ and mix thoroughly.
10. Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade these reagents.
11. Do not allow the plate to dry out between steps.
12. All products are carefully validated; however due to the variability encountered in biological sample matrices, the possibility of interference or sample matrix effects cannot be excluded.

KIT CONTENTS & STORAGE

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

Part/Description	Storage of opened/reconstituted material
Q-Plex™ Array Microplate REF 112352BV 1 arrayed and blocked 96-well polystyrene microtiter plate	2-8°C until kit expiration
Wash Buffer Concentrate (20X) REF 101158GR Liquid, 50 mL/vial of a concentrated solution of buffered surfactant	
Sample Diluent REF 101104HU Liquid, 10 mL/vial of a buffered protein solution with heterophilic antibody and rheumatoid factor blockers and preservatives	
Detection Mix REF 112356BV Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives	
Calibrator REF 112355BV Lyophilized, recombinant antigens in a buffered protein base	2-8°C until kit expiration Discard unused reconstituted calibrator.
Streptavidin-HRP REF 101173GR Liquid, 6 mL/vial of streptavidin-conjugated horseradish peroxidase	Do not expose to UV light. 2-8°C until kit expiration
Substrate A REF 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 B+ REF 101120GR Liquid, 3.5 mL/vial	
Plate Seals (3) REF 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 (20-200 µL) and/or single channel pipettes (20-1000 µL) with appropriate 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 Q-View™ Software
5. Microplate shaker capable of 500 RPM
6. Deionized water
7. Microplate washer (An optional multichannel pipette protocol is provided in Appendix A)
8. 1 L graduated cylinder for the preparation of wash buffer.

ASSAY PREPARATION

1. Install Q-View Software on any computers that will be used for analysis or operating a Q-View Imager.
2. Set up the imager. For imager-specific instructions, see www.quansysbio.com/manuals.
3. Set the microplate shaker to 500 RPM.
4. Allow all kit components to come to room temperature (20-25°C) prior to use.
5. Prepare Wash Buffer: Place 50 mL of Wash Buffer Concentrate (20X) into 950 mL deionized water and mix thoroughly.
6. Prepare Calibrator: Reconstitute lyophilized Calibrator using Sample Diluent with the volume specified on the In-Kit Certificate of Analysis accompanying the kit. Allow to sit for 5 minutes. Mix thoroughly. Use prepared Calibrator within 30 minutes.
7. Prepare chemiluminescent substrate: 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

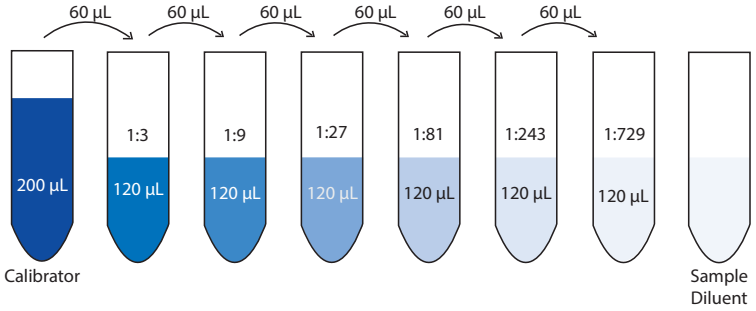
Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed in the previous sections. It is recommended that all calibrators, samples, and controls be assayed in duplicate.

1. Using the previously prepared Calibrator (see "Assay Preparation" step 6), prepare an 8-point calibration curve (7 points plus 1 blank) in either polypropylene tubes or a polypropylene 96-well plate (see "Materials Required but Not Supplied" for example tubes and plates).

    a. Pipette 200 µL of prepared Calibrator into the first tube or well.

    b. Pipette 120 µL of Sample Diluent into the other 7 tubes or wells.

    c. Transfer 60 µL of undiluted prepared Calibrator from the first tube or well into the second, mix thoroughly, and repeat the transfer from tube to tube or well to well for 5 more points, leaving the last tube or well without any prepared Calibrator. This process is diagrammed below. The undiluted prepared Calibrator serves as the high point of the calibration curve. The Sample Diluent serves as the blank.



2. Prepare samples by diluting 1:2 (1 part sample to 1 part 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.

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, use the prepared sample diluent to dilute your samples further.

3. Add 50 µL per well of the prepared calibration curve and samples to the Q-Plex™ Array Microplate. Load all samples and calibration curve to the plate within 10 minutes.
4. Cover the plate with a plate seal and incubate on a plate shaker set to 500 RPM for 120 minutes at room temperature (20-25°C).
5. Wash the plate 3 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 60 minutes at room temperature (20-25°C).
7. Wash the plate 3 times (see Appendix A).
8. 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).
9. Wash the plate 6 times (see Appendix A).
10. Add 50 µL per well of previously prepared substrate (see "Assay Preparation" step 8). 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.
12. Open Q-View Software, create or open a project, and click Acquire Image.
13. Use the chart below to determine the recommended imaging time based on imager model:

Imager Model	Recommended Imaging Time
Q-View™ Imager LS	270 seconds
Q-View™ Imager Pro	300 seconds

14. 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 > Q-View Software Manual**.

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

ANALYZING A Q-PLEX™ IMAGE

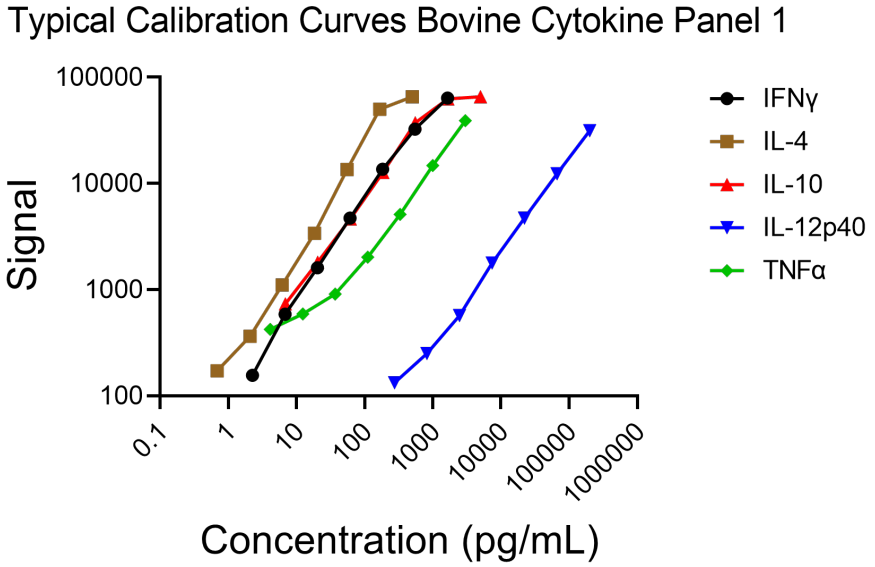
The following summarizes a general workflow for analyzing a Q-Plex image in Q-View Software. Each of these steps is described in greater detail in the Q-View Software and Imager manuals, viewable at www.quansysbio.com/support or within Q-View Software under **Support > Q-View Software Manual**.

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, calibrators, controls, or negatives (blank), 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 **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.

PERFORMANCE CHARACTERISTICS

Calibrator Curves

Expected signal for calibrator curves on Q-Plex assays varies depending on the imager model used and, to a lesser extent, between kit lots. Using a Q-Plex kit on a Q-Plex imager without negative subtraction turned on, the minimum signal observed will be around 100 and the maximum can be up to 65535. Below are examples of calibrator curves demonstrating the types of curves you may see on your kit. If you have concerns about your assay performance, please contact support at 1-888-QUANSYS (1-888-782-6797) or at support@quansysbio.com.



Typical Data

Representative data from one lot of kits are shown below to demonstrate typical calibrator curve performance. See below for explanation of data calculations.

Analyte	Units	ULOQ	LLOQ	LLD	Intra-Assay Precision (%CV)	Inter-Assay Precision (%CV)	Linearity Range	Linearity Average
IFNγ	pg/mL	1155	2.94	1.47	5%	14%	81-145%	110%
IL-4	pg/mL	350	0.89	0.45	3%	12%	72-140%	107%
IL-10	pg/mL	1166	8.92	4.46	3%	10%	84-137%	108%
IL-12p40	pg/mL	140000	356	178	7%	12%	71-136%	100%
TNFα	pg/mL	2100	16.05	8.03	4%	13%	93-145%	115%

Sensitivity

The upper limit of quantitation (ULOQ) is the highest concentration at which the calculated recovery of the analyte remains within 80-120% of the known value.

The lower limit of quantitation (LLOQ) is the lowest concentration at which the calculated recovery of the analyte remains within 75-125% of the known value and the CV of the calculated concentration is <25%.

The lower limit of detection (LLD) is the concentration that is two standard deviations above the average signal of twenty blank (zero) replicates.

Precision

Intra-Assay Precision – 3 samples containing and/or spiked with various concentrations of each analyte were tested 20 times on 1 plate to assess intra-assay precision. The average %CV of all samples is reported.

Inter-Assay Precision – 3 samples containing and/or spiked with various concentrations of each analyte were tested on at least 10 individual assays by at least 3 users. The average %CV of all samples is reported.

Linearity/Parallelism

Samples containing and/or spiked with various concentrations of each analyte were diluted with sample diluent at dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and assayed. The highest, lowest, and average dilution recovery are reported.

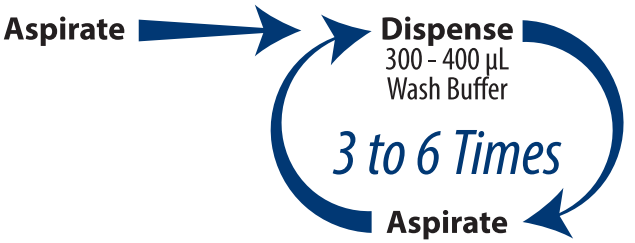
Specificity

Specificity of the array was assessed by testing each recombinant antigen at the concentration of the highest calibrator in the multiplexed assay. The cross-reactivity of all other antigens on each assay was less than 1%.

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 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 during wash steps

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 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

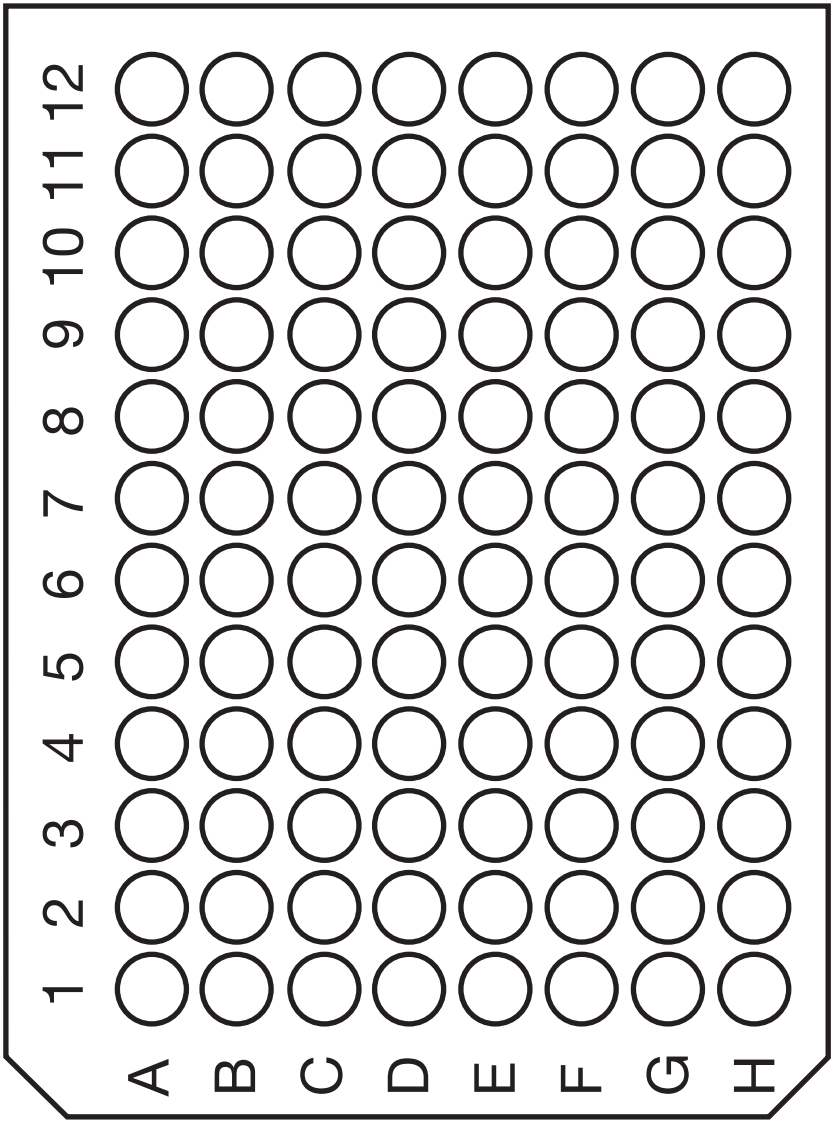
1. Install Q-View Software (page 8).
2. Set up the imager (page 8).
3. Set up microplate washer (page 16) and shaker (page 8).
4. Reconstitute and prepare reagents (page 8).

Running the Assay

5. Prepare the calibration curve using the prepared Calibrator and Sample Diluent according to the In-Kit Certificate of Analysis (page 9).
6. Prepare the samples with Sample Diluent (page 9).
7. Load the calibration curve and samples onto the plate. Shake for 120 minutes (500 RPM) at room temperature (page 10).
8. Wash the plate 3 times, add the Detection Mix, and shake for 60 minutes (500 RPM) at room temperature (page 10).
9. Wash the plate 3 times, add the Streptavidin-HRP, and shake for 20 minutes (500 RPM) at room temperature (page 10).
10. Wash the plate 6 times and add the prepared Substrate (page 10).
11. Capture and analyze image of the plate (page 11).

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 1-888-QUANSYS (1-888-782-6797) or at support@quansysbio.com.

PLATE DIAGRAM



NOTES

Q-Plex™ ARRAY

For technical support, email: support@quansysbio.com

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