Q-Plex™
Human Cytokine
IR (9-plex)

FOR RESEARCH USE ONLY
Catalog Number: 130251HU

Version 1.4
### Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Important Precautions</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Graphical Representation</td>
<td>6</td>
</tr>
<tr>
<td>Array Platform</td>
<td>6</td>
</tr>
<tr>
<td>Array Performance</td>
<td>7</td>
</tr>
<tr>
<td>Kit Contents</td>
<td>8</td>
</tr>
<tr>
<td>Required Materials</td>
<td>8</td>
</tr>
<tr>
<td>Video Manual</td>
<td>9</td>
</tr>
<tr>
<td>Setup of LI-COR® Odyssey® or Aerius® Infrared Imaging System</td>
<td>9</td>
</tr>
<tr>
<td>Setup of Plate Washer</td>
<td>9</td>
</tr>
<tr>
<td>Kit Component Reconstitution &amp; Stability</td>
<td>10</td>
</tr>
<tr>
<td>Procedure</td>
<td></td>
</tr>
<tr>
<td>Part 1-2</td>
<td>11</td>
</tr>
<tr>
<td>Part 3</td>
<td>12</td>
</tr>
<tr>
<td>Part 4-9</td>
<td>13</td>
</tr>
<tr>
<td>Part 10-12</td>
<td>14</td>
</tr>
<tr>
<td>Appendix A</td>
<td>15</td>
</tr>
<tr>
<td>Troubleshooting</td>
<td>16</td>
</tr>
<tr>
<td>Abbreviated Protocol</td>
<td>18</td>
</tr>
</tbody>
</table>

---

Quansys Biosciences is dedicated to the development of protein arrays to aid researchers and clinicians in better understanding, diagnosing, and treating disease for the betterment of people’s lives. Previous tests that have been developed include arrays for the quantitation of cancer markers, auto-reactive antibodies, Pneumococcal serotype-specific antibodies, cardiac markers, pituitary hormones, human cytokines, mouse cytokines, human chemokines, mouse chemokines, adipokines, angiogenesis factors, and obesity factors.
Important Precautions

Read all of the Instructions before beginning test.
1) Be sure that your LI-COR® Odyssey® or Aerius® Infrared Imaging System is set at the optimal settings and working properly before scanning (see page 9).

2) One key to running an optimal assay is thorough plate washing. This is accomplished by carefully following the wash instructions (See Appendix A). The most critical wash step is the final wash following the IRDye® 800CW Streptavidin incubation before scanning. It is vital that all unbound IRDye® 800CW Streptavidin and residual wash buffer is removed from the wells. Inadequate washing can result in high background/nonspecific signal, rendering quantification difficult.

3) Do not mix any components of this kit with the components from other kits, unless the instructions state otherwise.

4) When comparing results from this kit to other platforms, it is recommended that the user test a population of samples on both platforms. This is to validate to accuracy between the platforms.

5) Storage: Always keep the unopened kit containing the 96-well plate, Antigen Standard, Detection Mix, IRDye® 800CW Streptavidin, IRDye® 800CW Dilution Buffer, Sample Dilution Buffer, and Wash Buffer refrigerated at 4°C while not in use. After opening the kit and reconstituting the reagents, observe the stability recommendations stated on page 10 (Kit Component Reconstitution).

6) Prevent contamination by following instructions exactly, using properly functioning equipment and practicing aseptic techniques.

7) Do not expose the kit components to temperatures greater than 26°C.

8) Perform the test at room temperature: 20°-25°C.

9) Use new pipette tips whenever dispensing a new component.

10) Keep plate sealed until use. If using plates for multiple tests, keep unused wells sealed with plate covers supplied in kit.
Introduction

Quansys Q-Plex™ Human Cytokine Array - IR

The immune system is a dynamic symphony of many cell types employed in host protection, regeneration, and regulation. To work in concert, the immune system must communicate with exactness. Cytokines, hormone-like proteins produced by immune cells, act as the messengers of communication. Through cytokines, the immune system regulates stem cell differentiation, inflammation, the type of immune response executed, angiogenesis, and many other events. Because of these various activities, cytokines are also involved in progression and pathogenesis of most diseases.

Most cytokines perform multiple functions and often share the same receptors. A single cytokine can elicit one effect on one cell type, and a completely different effect on another cell type, then induce a completely different response when administered concomitantly with other cytokines. This diversity of activity is what allows the immune system to control a great variety of events with a relatively small repertoire of signaling agents. As a result, the profile of type and concentration of cytokines present at a specific time is critical to receiving the desired function. Because these profiles define what is happening in the immune system, and because of cytokine involvement in both disease pathogenesis and disease recovery, the monitoring of their levels is an invaluable tool in understanding, diagnosing, and monitoring disease.

Quansys multiplex ELISA Technology

Quansys Q-Plex™ Technology is based on tried and proven sandwich (capture) ELISA techniques. This process uses two antibodies. One antibody is first bound to a solid phase (i.e. micro-well plate). The sample is incubated on the surface, allowing the target analyte and bound antibody to associate. Following this, each well is incubated with a biotinylated secondary antibody. IRDye® 800 CW Streptavidin is then added and binds to the biotinylated antibody. This reaction is detected by monitoring the response of the dye, which is proportional to the amount of target analyte present in the sample.

Conventional ELISAs, though allowing the researcher to test many samples at one time, can test only one type of analyte in a single test. This makes testing multiple analytes costly with respect to time, reagents, and sample volume. These factors and the importance of profiles when working with multiple analytes have prompted many researchers to develop assays in multiplex formats. These formats have included fluorescent bead-based assays and slide-based protein arrays. These methodologies, in contrast to the conventional ELISA, allow the researcher to test many analytes at one time, but test only one or a few samples at a time, reducing the throughput of the assay.

The arrays using Quansys’ Q-Plex™ Technology are fully quantitative ELISA-based tests where up to 25 distinct capture antibodies have been adsorbed to each well of a 96-well plate in a defined array. Using as little as 30 µl of sample, up to 84 different samples can be assayed (assuming 12 wells are used for constructing a standard curve) for all 25 unique analytes in less than 2.5 hours. Sensitivity is system dependent. It typically ranges between 30 pg/ml to less than 1 pg/ml. All of the antibodies used in the Q-Plex™ arrays have been subject to a rigorous and comprehensive cross reactivity protocol and verified to be non-cross reactive with any other system on the array.

Quansys’ Q-Plex™ arrays are invaluable tools for the simultaneous detection of multiple analytes, providing a platform that overcomes the shortfalls of both conventional assays and predecessor multiplex assays to provide the researcher an easy and cost effective means of simultaneously exploring the complexity and range of many biological analytes.
Graphical Representation

Array Platform

Each well has 9 spots. The diagram below depicts the chemokine location in each well of the 96-well plate.
Array Performance

**Sensitivity:**
The Lower Limits of Detection (LLD) were determined for each system. The LLD is the mean of the background plus 2 standard deviations of the background.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Range (pg/ml)*</th>
<th>LLD (pg/ml)**</th>
<th>Standard (pg/ml)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>1100-1.51</td>
<td>1.44</td>
<td>1100</td>
</tr>
<tr>
<td>IL-1β</td>
<td>7500-10.29</td>
<td>1.56</td>
<td>7500</td>
</tr>
<tr>
<td>IL-2</td>
<td>2400-3.29</td>
<td>1.46</td>
<td>2400</td>
</tr>
<tr>
<td>IL-4</td>
<td>325-0.45</td>
<td>0.31</td>
<td>325</td>
</tr>
<tr>
<td>IL-6</td>
<td>2300-3.16</td>
<td>2.74</td>
<td>2300</td>
</tr>
<tr>
<td>IL-10</td>
<td>1500-2.06</td>
<td>0.66</td>
<td>1500</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>70000-96.02</td>
<td>4.51</td>
<td>70000</td>
</tr>
<tr>
<td>INFγ</td>
<td>1000-1.37</td>
<td>1.47</td>
<td>1000</td>
</tr>
<tr>
<td>TNFα</td>
<td>5000-6.86</td>
<td>0.45</td>
<td>5000</td>
</tr>
</tbody>
</table>

*If standard curves that approach the LLD for the chemokines are desired, it is recommended that the user continue 2-fold dilutions of the Antigen Standard.

**Please note that these LLD values are typical QC results. The LLDs for any released lot will vary slightly depending upon the specific lot.

***This is the concentration of the Antigen Standard when reconstituted with 300 μl. This will be the concentration of the high point of the standard curve.

For assay performance statistics, please review the Certificate of Analysis that accompanied your kit.
Kit Contents

1) 96-Well Plate: Pre-spotted and pre-blocked 96-well plate with 9 different cytokine capture antibodies in each well.
   a. To view the quality assurance image, please email us your plate code at info@quansysbio.com.
2) Antigen Standard
3) Detection Mix
4) IRDye® 800CW Streptavidin
5) IRDye® 800CW Dilution Buffer
6) Sample Diluent
7) Wash Buffer 20X
8) Plate Seals (2)

Required Materials

These items are required, but not included in the kit.

1) LI-COR® Odyssey® or Aerius® Infrared Imaging System
2) 8 or 12 channel pipette (20-200 µl) and/or one channel pipette and tips
3) Automatic plate washer, or Multi-channel pipette
4) Orbital shaker
5) Paper towels
6) Kimwipes® (or equivalent)
7) 70% ethanol
8) Quansys Q-View™ Software, Array-Pro Software, or similar software capable of array analysis
9) Deionized H₂O
Video Manual

1) A video demonstration on each step of the assay is available to download at www.quansysbio.com.
2) If a high-speed internet connection is not available, please contact us for a free copy. Toll-free 1-888-782-6797.

Setup of LI-COR® Odyssey® or Aerius®

This must be optimized before beginning the test.

Optimization of the LI-COR® Odyssey® or Aerius® settings must be performed. The following settings are recommended for initial testing.

<table>
<thead>
<tr>
<th>Settings</th>
<th>Odyssey®</th>
<th>Aerius®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>84 µm</td>
<td>50 µm</td>
</tr>
<tr>
<td>Quality</td>
<td>Lowest</td>
<td>Lowest</td>
</tr>
<tr>
<td>Focus Offset</td>
<td>3.9-4.0</td>
<td>3.4-3.8</td>
</tr>
<tr>
<td>Microplate Box</td>
<td>Checked</td>
<td>Checked</td>
</tr>
<tr>
<td>Channel</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>Intensity</td>
<td>7-10 (Rec. 8.5)</td>
<td>9-12 (Rec. 10.5)</td>
</tr>
<tr>
<td>Origin</td>
<td>X=0, Y=0</td>
<td>N/A</td>
</tr>
<tr>
<td>Size</td>
<td>Width=13, Height=9</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Optimization of the intensity setting and focus offset should be performed to obtain the best signal to noise ratio and the largest difference between the lowest standard dilution point and the zero standard point. Due to variability of cytokine levels in samples, multiple scans of differing intensities are recommended.

For more details on settings and setup, please contact Quansys Tech Support at 1-888-782-6797 or email at techsupp@quansysbio.com.

Setup of Plate Washer

1) Determine the method of washing you are going to use.
   a. Automatic plate washer (see Appendix A)
   b. Multi-channel pipette (see Appendix A)
2) Each method will effectively wash the plate. Choose the method most convenient for your lab.
Kit Component Reconstitution & Stability

Note: Store unopened kit at 4°C until ready to use.
Follow the suggested storage guidelines after opening the kit and reconstituting the reagents.

1) Sample Diluent
   a. Human serum and plasma samples must be diluted at least 1:2 prior to use to prevent interference.
   b. Cell culture samples can be run undiluted.
   c. Store unused Sample Diluent at 4°C. Good for 1 year.

2) Antigen Standard
   This will be used either as a control or to construct a standard curve.
   a. To the vial labeled Antigen Standard add 300 µl Sample Diluent.
   b. Vortex the vial until the lyophilized Antigen Standard is fully reconstituted.
   c. Store unused Antigen Standard at -20°C. Good for 1 week.

3) Detection Antibody Mix
   a. Add 3.5 ml of deionized water to the lyophilized Detection Mix.
   b. Vortex the vial until the lyophilized Detection Mix is fully reconstituted.
   c. Store unused Detection Antibody Mix at 4°C. Good for 1 month.

4) IRDye® 800CW Streptavidin
   a. Reconstitute vial of IRDye® 800CW Streptavidin with 50 µl deionized water.
   Note: We recommend reconstituting the IRDye® 800CW Streptavidin 24-hours before running the test.
   b. Reconstitute vial of IRDye® 800CW Dilution Buffer with 4 ml of deionized water.
   c. Add 4 µl of the IRDye® 800CW Streptavidin to the 4 ml of IRDye® 800CW Dilution Buffer.
   d. Vortex the vial until the dye and buffer are completely mixed.
   e. Store unused IRDye® 800CW Streptavidin at 4°C. Good for 1 year.
   f. Do not allow IRDye® 800CW Streptavidin to be exposed to light for extended periods of time.

5) Wash Buffer
   a. Mix 50 ml of 20X Wash Buffer with 950 ml of deionized water and thoroughly mix.
   b. Store unused wash buffer at 4°C for up to 1 year.
   c. Some precipitation may occur over time. This will not affect assay performance.
Procedure

Note: It is recommended that reagents be warmed to room temperature before they are added to the plate, with the exception of the Wash Buffer, which should be kept at 4°C.

1) Eight Point Standard Curve Preparation
   a. Duplicate standard curves will require at least 60 µl of each dilution point. This protocol will prepare 80 µl to ensure adequate volume.
   b. Prepare standards by adding 80 µl of Sample Diluent to six separate vials. Then transfer 40 µl from the Antigen Standard to the first vial containing 80 µl of Sample Diluent and mix thoroughly. This will be the 1:3 point on the standard curve.
   c. Make the 1:9 point for the standard curve by removing 40 µl from the 1:3 dilution and mixing it with the next vial of 80 µl of buffer.
   d. Continue 1:3 serial dilutions four more steps until the last point is at a final dilution factor of 1:729. This vial will have a total ending volume of 120 µl.

   ![Diagram of standard curve preparation](image)

2) Sample Preparation
   a. All samples must be diluted at least 1:2 in Sample Diluent.
   b. If you anticipate that your sample concentrations will be higher than the ranges on the standard curves, use the Sample Diluent to dilute the samples further.
3) Addition of Samples to the 96-Well Plate
   a. The standard curve can be placed anywhere on the plate, however, in reference to the plate diagram below, the process of the standard curve addition will be explained.
   b. For this example, the standard curve will be run in rows G and H of the plate. Place 30 µl of the Antigen Standard in the first wells of the rows (G1 and H1). These wells also serve as a positive control in that they should always have a bright response. In the following rows (G2-G7 and H2-H7), place 30 µl from each of the dilutions prepared in step 1. In the last wells (G8 and H8), place 30 µl of the Sample Dilution Buffer. These wells will be used to determine the background of the assay and also serve as a negative control.
   c. In each well place 30 µl of sample into its appropriate location. (See diagram below to plan the location of samples.)
   d. Once the standard curve and samples have been added to the plate, cover the plate with a plate seal, then place the plate on an orbital shaker at 120 RPM for 1 hour at room temperature.
   e. If you choose not to run the entire plate at this time, make sure the unused wells are well covered with a plate cover supplied in the kit to insure the plate doesn’t dry out. The remaining reagents can be stored at 4°C for 1 week except for the antigen standard that must be frozen after reconstitution for future use.

Note: Make sure to add samples and standard curve in a timely manner. Do not take longer than 10 minutes to add them to the plate. To help, you may want to prepare them in a different plate and transfer when ready.

Caution: Do not touch the pipette tips on the side of the well when adding sample or standard curve.
Procedure cont.

4) Washing of the Plate
   a. Using the predetermined wash method, wash the plate three times.
      (For more information, see Appendix A)

5) Addition of Detection Mix
   a. Add 30 µl of the previously reconstituted Detection Mix to each of the
      wells that received sample, whether it was the standard curve, control, or unknown samples.
   b. Cover the plate with a plate seal and place the plate back on the
      orbital shaker at 120 RPM for 1 hour at room temperature.

   Caution: Take caution in washing and adding reagent to the wells as to not
   cause bubbles. Bubbles will create difficulties when scanning and analyzing the plate.

6) Removal and washing of the Detection Mix from the Plate
   a. Using the predetermined wash method, wash the plate three times.

7) Addition of IRDye® 800CW Streptavidin to the Plate
   a. Add 30 µl of the previously diluted IRDye® 800CW Streptavidin to
      each of the wells that received sample, whether it was the standard curve or unknown samples.
   b. Cover the plate with a plate seal and place the plate back on the
      orbital shaker at 120 RPM for 15 minutes at room temperature.

   Caution: Do not touch the pipette tips on the side of the well when adding
   the IRDye® 800CW Streptavidin.

8) Rinsing the remaining IRDye® 800CW Streptavidin from the Plate
   a. Using the predetermined wash method, wash the plate six times.
   b. Rinse the wells by hand with 100-200µl of water.
   c. Decant the wells immediately.

9) Drying the Plate
   There are two methods to dry the plate before imaging.
   a. Centrifugation. Place the plate face down upon a dry paper towel.
      Centrifuge the plate at 300g for 2 minutes. This method is recommended for optimal results.
   b. Air Dry. Vigorously flick the water out on a paper towel on the lab bench. Ensure that all liquid is flicked out of the plate. Place the plate in open air and allow to air dry for 10 minutes at room temperature.
Procedure cont.

10) Cleaning the bottom of the Plate
   a. Using Kimwipes® (or equivalent) and 70% ethanol, clean the bottom of the plate and scanner surface to eliminate background caused by dust.

11) LI-COR® Odyssey® or Aerius® Scanning of the Plate
   a. After setting up the imager perform an initial scan. Adjust the image intensity up or down depending upon the brightness of each system. The optimal intensity assures that the majority of the system’s high points in the standard curve approach saturation (65500 pixel intensity) without saturation of any second dilution point.
   b. Multiple scans of varying intensities are recommended. It may be necessary to analyze multiple scans to obtain the best results for each system.

12) Quansys Q-View™ Software
   Please visit www.quansysbio.com to download a copy of the Quansys Q-View™ Software. Please contact Quansys for a user name and password to download software.

13) Additional Services
   Please contact Technical Support at 1-888-782-6797 or email techsupp@quansysbio.com.
Appendix A

1) Automatic Plate Washer
   a. Connect the prepared wash buffer to your automatic plate washer.
   b. Run 1-2 prime 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.)
   c. Make sure that the plate washer is able to wash a plate (dispense and aspirate 400 µl of
      wash buffer) three times and six times. Both types of washes are used in the protocol.
   d. No soak or shaking cycles are needed.
   e. Prime the plate washer one time before each wash step.
   f. Inspect the plate for residual wash solution. If residual wash solution remains,
      vigorously tap the upside-down plate against a paper towel on a hard surface to remove
      any excess wash solution.

2) Multichannel Pipette
   a. After each incubation, aggressively flick the solution out of the plate over a waste
      container before starting the wash protocol.
   b. Pour the prepared Wash Buffer into a trough or tray.
   c. Using a multichannel pipettor, pipette 200-400 µl of Wash Buffer into each of the wells
      used in the test.
   d. Aggressively flick the wash buffer out over a waste container.
   e. This washes the plate one time.
   f. Inspect the plate for residual wash solution. If residual wash solution remains,
      vigorously tap the upside-down plate against a paper towel on a hard surface to remove
      any excess wash solution.
Troubleshooting

1) No Response
   a. Did the positive control light up?
      1) No - IRDye® 800CW Streptavidin was omitted, added incorrectly, set at too low of intensity, or was faulty and did not work. Check working dilution and expiration date.
      2) Yes
   b. Was sample added?
      Review the procedure steps and see if anything was overlooked.
      1) Incubation times
      2) Correct buffers used
      3) Correct reagents used

2) Low or Saturated Response
   a. Low Response
      1) Was the proper volume used to reconstitute the detection mix? If too much water was used, then the detection mix will be too dilute and produce a low response.
      2) Were the samples diluted too much? Diluting them further than the recommended amount may produce a low response.
      3) Has this plate been used in a previous test where the unused wells received a wash? This may prematurely diminish the response because the capture antibodies received extra washes.
      4) Was the focus offset optimized? At what intensity was the plate scanned? Too low of an intensity setting will produce a low response.
      5) Were the wells still wet? If any wells still contain liquid, the response will be diminished. Continue taking images (typically three) until the image is of acceptable response.
   b. Saturated Response
      1) At what intensity was the plate scanned? Too high of an intensity setting will produce a saturated response.
      2) Was the proper volume used when mixing the IRDye® 800CW Streptavidin?
      3) Were the proper sample dilutions used? If the samples are too concentrated, it will result in a saturated response.

3) Background
   a. Have the kit reagents expired? Old reagents may create a higher background.
   b. Was the buffer supplied with the kit used to dilute the samples? Were the samples diluted at least 2-fold in Sample Diluent?
      The assay has been optimized to use the buffers supplied with the kit.
   c. Were the kit reagents left at a temperature other than 4°C for an extended amount of time? If so, the reagents may degrade and cause high background.
   d. Was there dust on the plate bottom or on the glass surface of the scanner?
   e. Inadequate washing caused by too few repetitions.
   f. Was the plate washed with water instead of Wash Buffer?

4) Poor Standard Curve
   a. Were the dilutions for the standard curve made correctly? Double-check all the calculations to make sure there was no error.
   b. Pipettor error-miscalibrated or malfunctioning pipettor.
5) Replicate Variability
   a. If the replicates do not show an identical profile (large differences in intensity), the samples may have been placed in the wrong wells.
   b. Also, double check the tips on the pipettor. If they are not on correct or if the pipette is not calibrated, unacceptable variation can occur.

6) Random Fluorescence
   a. If a crescent-shaped signal shows up in some of the wells, then most likely the pipette tips touched the side of the well when adding dye.
   b. Check the automatic plate washer to see if any of the channels are clogged.
   c. If washing by hand, make sure to completely fill each of the wells with wash solution.
   d. If washing by hand, make sure to flick out the excess wash solution completely.

7) Failed Control
   a. Positive Control
      1) Was the Antigen Standard, Detection Mix, and IRDye® 800CW Streptavidin added to the positive control wells or the top point of the standard curve before imaging the plate?
   b. Negative Control
      1) If the plate is not properly washed, residual dye will remain in the wells and may cause the negative control to light up.
Abbreviated Protocol

1) Set up the LI-COR® Odyssey® or Aerius® Infrared Imaging System.

2) Determine the method of plate washing and prepare the wash buffer.

3) Reconstitute the lyophilized vials by adding the indicated amount of dH₂O.

4) Prepare the standard curve by doing 1:3 serial dilutions of the Antigen Standard with the Sample Diluent.

5) Prepare the samples.

6) Add the standard curve to the bottom left hand corner of the plate in rows G & H.

7) Add the samples to the plate.

8) Incubate the samples and standard curve for 1 hour.

9) Wash the plate 3 times.

10) Add the Detection Mix to every used well on the plate.

11) Incubate the Detection Mix for 1 hour.

12) Wash the plate 3 times.

13) Add the IRDye® 800CW Streptavidin to every used well on the plate.

14) Incubate the IRDye® 800CW Streptavidin for 15 minutes.

15) Wash the plate 6 times.

16) Hand rinse the plate with water, then decant the water.

17) Dry the plate.

18) Clean the bottom surface of the plate and the scanner surface.

19) Scan the plate on LI-COR® Odyssey® or Aerius® Infrared Imaging System.