

## Instructions for using the Quansys Q-Plex™ Calibration Kit - IR

Thank you for your interest in the Quansys Q-Plex™ Array. This Calibration kit is a tool designed for researchers to evaluate the Q-Plex™ Array platform in their labs.

### Kit Contents for the Calibration Kit- IR

- |                                 |                    |
|---------------------------------|--------------------|
| 1) 96-well Calibration Plate    | 4) Wash Buffer 20X |
| 2) IRDye® 800CW Streptavidin    | 5) Plate Seals (2) |
| 3) IRDye® 800CW Dilution Buffer |                    |

It is our aim to help ensure each user is able to have a successful experience when using one of our arrays. In order to make this happen we highly recommend having an in-depth discussion with one of our technical support personnel. They can walk each user through the various steps of the array process, from sample preparation to adjusting the settings on the imager and analyzing the array image. Please contact us at 1-888-QUANSYS (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.

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

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.

### Secondary Calibration using Calibration Plate

- 1) Preparation of Wash Buffer
  - a. Add 50ml of the 20X Wash Buffer to 950 ml of deionized water in a clean sterile 1-Liter bottle.
  - b. Invert bottle to ensure sufficient mixing.
  - c. Determine the method of washing you are going to use.
    - 1) Automatic plate washer
    - 2) Multi-channel pipette
- 2) Open the Calibration Plate bag and remove the 96-well plate.
- 3) Locate the vials in your Calibration Kit labeled IRDye® 800CW Streptavidin and IRDye® 800CW Dilution Buffer.

- 4) 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.*
- 5) Reconstitute vial of IRDye® 800CW Dilution Buffer with 4 ml of deionized water.
- 6) Add 4µl of IRDye® 800CW Streptavidin to 4ml vial of IRDye® 800CW Dilution Buffer.
- 7) Vortex the vial until the dye and buffer are completely mixed.
- 8) Add 30µL of the liquid IRDye® 800CW Streptavidin diluent to each well of the calibration plate.
- 9) Put a plate cover over the plate and place it on an orbital shaker at 120 RPM for 15 minutes at room temperature.
- 10) Using the predetermined wash method, wash the plate six times.
- 11) Rinse the wells by hand with 100-200µl of water, then decant the wells immediately.
- 12) 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.
- 13) Using Kimwipes® (or equivalent) and 70% ethanol, clean the bottom of the plate and scanner surface to eliminate background caused by dust.
- 14) After setting up the imager perform an initial scan. Adjust the image intensity up or down depending upon the brightness of the spots.
- 15) Store unused kit components at 4°C.