

Instructions for using the Quansys Q-Plex™ Calibration Kit

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

Kit Contents for the Calibration Kit

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|------------------------------|--------------------|
| 1) 96-well Calibration Plate | 5) Substrate B |
| 2) Streptavidin-HRP 1X | 6) Plate Seals (2) |
| 3) Wash Buffer 20X | 7) Mouse Pad |
| 4) Substrate A | |

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 CCD Imager with use of Mouse Pad to ensure the camera is in focus

- 1) Set the f-stop at the lowest setting on the camera. (The aperture is fully opened.)
- 2) Open the imaging software and set to expose preview.
- 3) Using the provided mouse pad as a guide, open the imager box and place the mouse pad inside the field of view. Do not close the camera box. With the aperture fully opened, the mouse pad should be visible in the imaging software.
- 4) Adjust the zoom on the camera so that the image of the plate takes up 80% of the field of view in the imaging software.
- 5) Adjust the focus on the camera so that the individual spots on the plate are visible and clear in the imaging software.
- 6) Check the settings on the camera to make sure that it is not set to bin pixels. Binning pixels will bring the resolution of the camera down.
- 7) Make sure the imager box is completely light sealed, as the response is chemiluminescent.

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 (see Appendix A of user manual)
 - 2) Multi-channel pipette (see Appendix A of user manual)
- 2) Open the Calibration Plate bag and remove the 96-well plate.
- 3) Locate the vials in your Calibration Kit labeled Streptavidin-HRP 1X.
- 4) Add 30µL of the liquid Streptavidin-HRP 1X to each well of the calibration plate.
- 5) Put a plate cover over the plate and place it on an orbital shaker at 120 RPM for 15 minutes at room temperature.
- 6) Using the predetermined wash method, wash the plate six times. (For more information, see Appendix A of user manual.)
- 7) Empty the contents of Substrate A and mix with the contents of Substrate B in a separate container (not included).
- 8) Ensure that Substrate A and Substrate B are mixed well.
- 9) Add 40µL of the substrate mix to each well of the calibration plate.
- 10) Place the plate in your imaging system and capture an image.

- 11) Exposure times and small adjustments to the focus will need to be determined by taking multiple exposures until you can clearly distinguish bright spots in each well.
- 12) Store unused kit components at 4°C.