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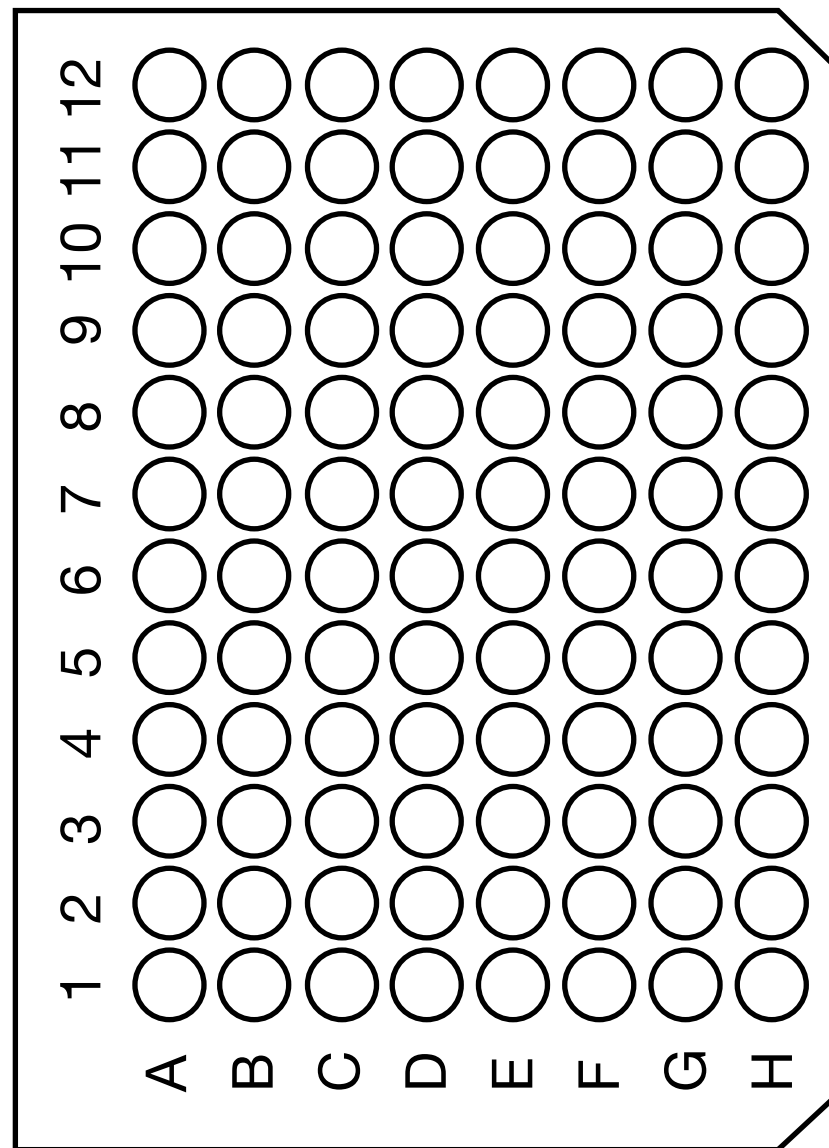
QUANSYS 
B I O S C I E N C E S

Quansys Q-Plex™ Array
Chemiluminescent

For Research Use Only Version 1.0

TABLE OF CONTENTS

| | |
|--|----|
| Important Precautions | 2 |
| Supported Imaging Systems | 2 |
| Kit Contents | 3 |
| Required Supplies | 4 |
| Video Manual | 4 |
| Kit Component Reconstitution And Stability | 4 |
| Choosing A Plate Washing Method | 5 |
| Automatic Plate Washer Method | 5 |
| Multichannel Pipette Method | 6 |
| Running The Assay | 7 |
| Acquiring An Image Using the Quansys Q-View Imager | 9 |
| Acquiring An Image By Importing An Image File | 10 |
| Analyzing The Image | 11 |
| Appendix A: Interferences And Compatibility | 12 |
| Appendix B: Alpha Innotech HD2 Setup | 13 |
| Appendix C: Alpha Innotech FC2 Setup | 14 |
| Appendix D: Bio-Rad VersaDoc 4000 Setup | 15 |
| Appendix E: Bio-Rad ChemiDoc XRS Setup | 18 |
| Appendix F: FujiFilm LAS 3000 Setup | 20 |
| Notes | 22 |
| Abbreviated Protocol | 25 |
| Plate Diagram | 26 |



ABBREVIATED PROTOCOL

- 1) Set up the camera using the included mouse pad as a guide.
- 2) Determine the method of plate washing and prepare the wash buffer.
- 3) Reconstitute the lyophilized vials by adding the indicated amount of diluent.
- 4) Prepare the standard curve by doing 1:2 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 Streptavidin HRP 1X to every used well on the plate.
- 14) Incubate the Streptavidin HRP 1X for 15 minutes.
- 15) Mix Substrate A and Substrate B together and allow to sit at room temperature
- 16) Wash the plate 6 times.
- 17) Add the mixed Substrate to every used well on the plate.
- 18) Image the plate at 1 and 4 minute intervals.

IMPORTANT PRECAUTIONS

Read all instructions before beginning test.

- 1) For research use only. Not for use in diagnostic procedures.
- 2) The kit should not be used beyond the expiration date on the kit label.
- 3) Do not mix or substitute reagents with those from other lots or kits.
- 4) If samples generate values higher than the highest standard, dilute the samples with sample diluent and repeat the assay.

SUPPORTED IMAGING SYSTEMS

The following is a list of imagers compatible for use with Quansys Biosciences' Q-Plex™ multiplex ELISA technology:

- 1) Quansys Q-View™ Imager (Cat # 104450GR) with Q-View™ Software.
Recommended for the acquisition of the chemiluminescent signal and quantification of spot intensity for the Q-Plex™ multiplex ELISA arrays.
- 2) Alternative imagers that are compatible with the Q-Plex™ multiplex ELISA array products.
 - Alpha Innotech: Fluorchem HD, SP, 8000, 8900, 9900, HD2, and FC2
 - For the HD2 camera and software set up instructions, see Appendix B.
 - For the FC2 camera and software set up instructions, see Appendix C.
 - Bio-Rad: Versa Doc 4000 or XRS
 - For the VersaDoc 4000 camera and software set up instructions, see Appendix D.
 - For the ChemiDoc XRS camera and software set up instructions, see Appendix E.

- Fujifilm: LAS-3000, LAS-3000 Mini
 - For the LAS-3000 camera and software set up instructions, see Appendix F.
- LI-COR: Aeries®, Odyssey®

3) Alternative imagers that may work but have not been tested with Q-Plex™ multiplex ELISA array products.

- Fujifilm: LAS-4000, LAS-4000 Mini
- Kodak: 4000MM, 2000MM, Gel Logic 100
- UVP: BioDoc-IT System, EC3 Darkroom

KIT CONTENTS

- 1) 96-well plate — multiplex array bound and blocked in each well of the plate
- 2) Antigen standard — lyophilized (300 µl)
 - a. When comparing results from this kit to other platforms, it is recommended that the user test the same antigen standards and samples on both platforms. This is to validate the accuracy of the standard from one platform to another.
- 3) Detection mix — lyophilized (3.5 ml)
- 4) Streptavidin HRP 1X (4 ml)
- 5) Substrate — for chemiluminescence. Do not mix until instructed.
 - a. Substrate A (2.5 ml)
 - b. Substrate B (2.5 ml)
- 6) Sample diluent (10 ml)
 - a. See Appendix A for information regarding compatible buffers and array inhibitors.
- 7) Wash buffer 20X (50 ml)
- 8) Plate seals (2)

REQUIRED SUPPLIES

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

- 1) 8- or 12-channel pipette (20-200 μ l) and/or 1-channel pipette and tips
- 2) Imaging system
- 3) Plate shaker

VIDEO MANUAL

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

KIT COMPONENT RECONSTITUTION AND STABILITY

Store unopened kit at 4°C until ready to use. After opening the kit and reconstituting the reagents, follow the suggested storage guidelines below.

- 1) Sample diluent
 - a. This solution comes mixed and ready for use.
 - b. Store unused sample diluent at 4°C. Good for 1 year.
- 2) Antigen standard
 - a. Add 300 μ l of sample diluent.
 - b. Mix gently until 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.
- b. Mix gently until fully reconstituted.
- c. Store unused detection antibody mix at 4°C. Good for 1 week.

4) Wash buffer

- a. Place 50 ml of the 20X wash buffer in a clean 1-liter bottle, then fill bottle to capacity with deionized water.
- b. Invert bottle to ensure sufficient mixing.
- c. Store unused wash buffer at 4°C. Good for 1 year.

5) Substrate A and substrate B

- a. Mix 2.5 ml of substrate A with 2.5 ml of substrate B.
- b. Mix gently to ensure homogeneity of the solution.
- c. Once the components of substrates A and B are mixed, they can be stored at room temperature and will be stable for 1 week. Do not store at 4°C after mixing.

6) Streptavidin HRP 1X

- a. This solution comes mixed and ready for use.
- b. Store unused streptavidin HRP 1X at 4°C. Good for 1 year.

CHOOSING A PLATE WASHING METHOD

Before running the assay, you should select and become familiar with a plate washing method. If you have an automatic plate washer, use the automatic plate washer method described below. If you do not have an automatic plate washer, follow the instructions for the multichannel pipette method.

Automatic Plate Washer Method

- 1) Connect the prepared wash buffer to your automatic plate washer.

e. Select the “Return” button on the software to close the focusing window.

3) Image Acquisition

a. When the plate is ready to image, press the “Start” button.

b. After the plate has imaged invert the image to black with white spots by clicking on “View” then selecting “Positive Gray” in the drop down menu.

c. Save the image by pressing the “Save” button. In the new window select “16 bit linear tiff” in the “Save as type” drop down menu. Then type a name for the file and select “Save.”

d. Press the “Complete” button to allow the imager to take another image.

e. Take multiple images at different exposure times to ensure you get the best reading possible. Example exposure times are 20 seconds, 45 seconds, 60 seconds, 90 seconds, and 120 seconds.

4) Image Optimization

a. Once the image is acquired, look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000-55,000 Pixel Intensity range, and on the second spot they should be in the 20,000-40,000 pixel intensity range.

i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then re-expose image of the plate for a shorter period of time (i.e. 20 seconds).

ii. If the spots on the high point of the curve in general fall below 40,000 PI, then re-expose the image for a longer period of time (i.e. 2 minutes).

2) 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.)

3) Make sure that the plate washer is able to wash a plate automatically (dispense and aspirate 300 μ l of wash buffer) three times and six times. Both types of washes are used in the protocol.

4) No soak or shaking cycles are needed.

5) Prime the plate washer one time before each wash step.

6) 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.

Multichannel Pipette Method

1) After each incubation, aggressively flick the solution out of the plate over a waste container before starting the wash protocol.

2) Pour the prepared wash buffer into a trough or tray.

3) Using a multichannel pipette, deposit 200-300 μ l 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 one time. When the procedure for running the assay calls for three and six washes, repeat steps 1-4 accordingly.

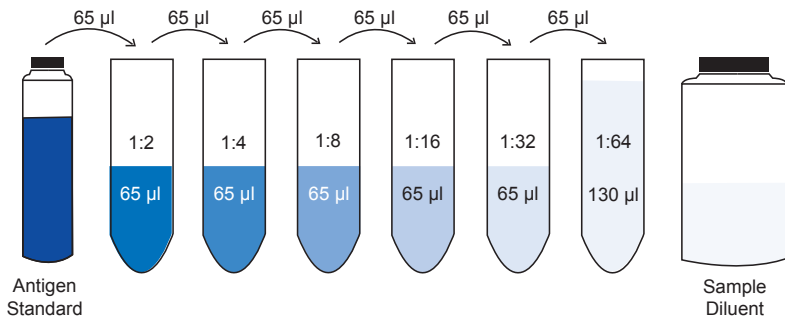
6) Inspect the plate for residual wash solution. If residual wash solution remains, vigorously tap the upside-down plate against a paper towel on a hard surface to remove any excess wash solution.

RUNNING THE ASSAY

The following procedure will guide you through the steps of running the assay.

1) Prepare eight point standard curve

- a. Pipette 65 μ l of sample diluent into each tube. Use the antigen standard to produce the dilution series below.
- b. Mix each tube thoroughly before the next transfer. The undiluted antigen standard serves as the high point of the standard curve. The sample diluent serves as the low or zero point of the standard curve.



2) Prepare samples

- a. All serum/plasma samples must be diluted at least 1:2 with sample diluent to minimize interference.
- b. If you anticipate that your sample concentration will be higher than the ranges on the standard curves, use the sample diluent to dilute your samples.
- c. Various sample types may be tested with this kit such as tissue culture supernate, serum, plasma and urine.

3) Add samples to the 96-well plate

- a. Add 30 μ l of sample and standard to each well. (See diagram on page 26 to plan the location of samples and standard.)

- c. Select "Export raw data," click on "Export," then click on "Save."

APPENDIX F: FUJIFILM LAS 3000 CAMERA AND SOFTWARE SETUP

1) File Setup

- a. Open the software on the computer.
- b. Under "Exposure Type" select "Precision" in the drop down menu.
- c. Under "Exposure Time" set the imager to take a 30-second image by selecting "Manual" then entering "30" in the first box and selecting "sec" in the second box.
- d. Under "Sensitivity" select "Standard" in the drop down menu.
- e. Ensure the box next to "Image Acquire & Digitize" is checked.
- f. Click on the "Method/Tray Position" button. In the window that appears, select "Chemiluminescence," and under tray position select "2." Then select "OK" to close the window.

2) Camera Setup & Focus

- a. Open the camera box and make sure the tray is in position "2."
- b. Place the Quansys mouse pad on the tray or other imaging target sheet and close the door.
- c. On the software select "Focus" and a new window appears. In the "Adjust" area, click up or down on the arrows until the writing or spots on the mouse pad are in focus.
- d. Remove the mouse pad from the imager and place the plate in the center of the tray. Look on the computer screen to make sure the plate is centered and straight in the imaging screen. Close the imager door when the plate is centered.

h. Replace the imaging target sheet with the plate, close the drawer, and make sure the plate is in the center of the imaging screen and straight.

i. Turn off the "Epi White" button on the cabinet, and select "freeze" in the software.

3) Image Acquisition

a. On "Step III-Acquire Image" on the software, change the exposure time to 30 seconds and select Manual Expose.

b. When the exposure is complete, convert the image into negative (black background with white spots).

i. Click Image, and new menu appears.

ii. Select Transform, and then check the box that says "Invert display."

iii. Click OK.

c. Save the image.

4) Image Optimization

a. Once the image is acquired, look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000-55,000 Pixel Intensity range, and on the second spot they should be in the 20,000-40,000 pixel intensity range.

i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then re-expose image of the plate for a shorter period of time (i.e. 1 minute).

ii. If the spots on the high point of the curve in general fall below 40,000 PI, then re-expose the image for a longer period of time (i.e. 3 minutes).

5) File Conversion

a. After acquiring the images, you need to convert them to Tiff files.

b. Click on "File" then "Export to Tiff image."

b. Cover the plate and place on a plate shaker for 1 hour at room temperature.

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

4) Wash the plate three times using one of the methods described previously (see page 5).

5) Add detection mix

a. Add 30 μ l of the Detection Mix to each of the wells.

b. Cover the plate and place on a plate shaker for 1 hour at room temperature.

6) Wash the plate three times using one of the methods described previously (see page 5).

7) Add Streptavidin HRP 1X

a. Add 30 μ l of the Streptavidin HRP 1X to each of the wells.

b. Cover the plate and place on a plate shaker for 15 minutes at room temperature.

8) Wash the plate six times using one of the methods described previously (see page 5).

9) Add substrate and image the plate

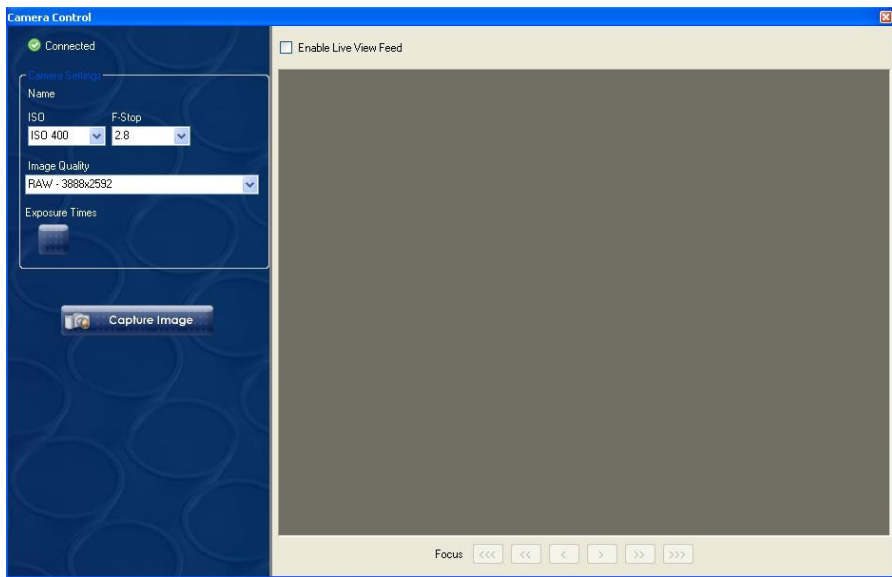
a. Add 40 μ l of substrate to each well.

b. Image the plate immediately after substrate is added for optimal results. Wait no longer than 15 minutes to commence imaging.

ACQUIRING AN IMAGE USING THE QUANSYS Q-VIEW IMAGER

These are instructions for using the Q-View Software to image your plate. To begin imaging a plate, open the Q-View Software.

Select **Image Acquisition > Imaging System** from the Navigation Bar or the **Capture Image** button on the Q-View Software main screen. A camera control window appears.



If the camera is recognized, “Connected” appears in Green in the top left corner of the dialog box. If the camera is not recognized, “Not Connected” appears in red. Please contact customer support at 1-888-QUANSYS for assistance in establishing a connection with the camera.

If the camera is connected, type the desired settings in the ISO, F-Stop, Exposure time and Image Quality fields. Recommended settings are as follows:

ISO: 400
F-Stop: 2.8

APPENDIX E: BIO-RAD CHEMIDOC XRS CAMERA AND SOFTWARE SETUP

1) File Setup

- a. Open the software on the computer.
- b. Click on “File,” then select “ChemiDoc XRS.”
- c. Under “Step I-Select Application” press the “Select” button, then select “Custom” and then “Create.”
- d. When the new window opens, name this custom setup as “Quansys 1X1 binning.” Under “Illumination” select “none,” and under “gain & binning” select “2X, 1X1” and click “OK.” (Now that this setting is saved you can use it again for future exposures. Instead of selecting “create” select “Quansys 1X1 binning.”)

2) Camera Setup & Focus

- a. On the software select “Live Focus.”
- b. On the cabinet press the plus button to open the aperture or iris all the way (the lowest number).
- c. Open the camera’s drawer, place the plate in the middle of the drawer, then close the drawer.
- d. Press the “Epi White” button on the camera cabinet
- e. Press the zoom buttons on the cabinet until the plate fills most of the screen on the computer.
- f. Replace the plate with the “imaging target sheet” or a mouse pad and close the door
- g. Press the focus buttons on the cabinet until the targets or lettering are in focus on the computer screen.

h. Close the cabinet door.

3) Image Acquisition

a. On “Step III-Set exposure time” on the software, change the exposure time to 30 seconds and select Acquire.

b. When the exposure is complete, convert the image into negative (black background with white spots).

i. Click Image, and new menu appears.

ii. Select Transform, and then check the box that says “Invert display.”

iii. Click OK.

c. Save the image.

4. Image Optimization

a. Once the image is acquired, look at the pixel intensity of the high points on the second dilution in the standard curve, and make sure they are not saturated. On average, most of the high points on the curve should be in the 45,000-55,000 Pixel Intensity range, and on the second spot they should be in the 20,000-40,000 pixel intensity range.

i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then re-expose image of the plate for a shorter period of time (i.e. 1 minute).

ii. If the spots on the high point of the curve in general fall below 40,000 PI, then re-expose the image for a longer period of time (i.e. 3 minutes).

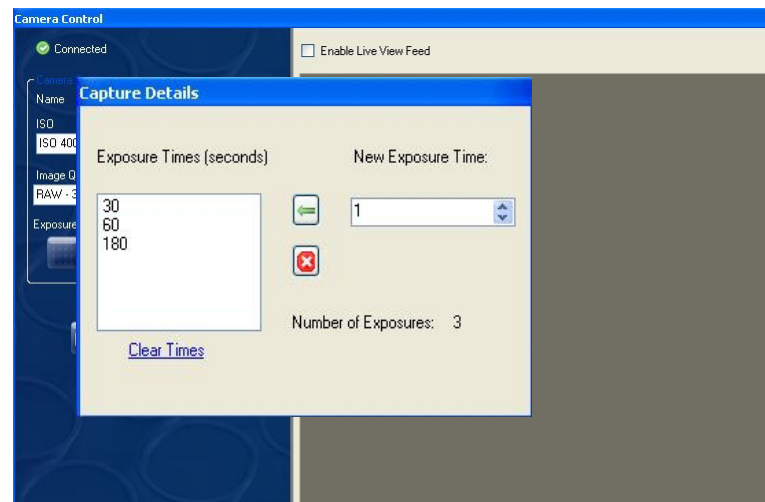
5. File Conversion

a. After acquiring the images, you need to convert them to Tiff files.

b. Click on “File” then “Export to Tiff image.”

c. Select “Export raw data,” click on “Export,” then click on “Save.”

The Exposure Times setting can be accessed by clicking on the Exposure Times button.



Exposure Times: These times can be modified to meet your specific assay, but 30, 60, and 180 second exposure times are recommended for most assays. Once set, one can exit the Exposure Times dialog by mousing outside of the dialog box.

Image Quality: **RAW**

Once the settings are adjusted accordingly, place the plate in the Imager and shut the door. Click the capture button. The image will then be displayed in the Q-View Software main screen.

ACQUIRING AN IMAGE BY IMPORTING AN IMAGE FILE

Q-View Software can process images in the following formats: CR2 (raw image files from Canon cameras), TIFF, JPEG, PNG and BMP. Users should be taking images from our supported imaging systems list (see page 2).

To acquire an image by importing an image file, select **Image Acquisition > Import Image** from the Navigation Bar or the **Import Image** button on the Q-View

Software main screen. An Open dialog box will appear. Navigate to the file you want and select **Open**.

The time to upload the image will vary depending on the image file type and size. Once acquired, the image will appear in the Q-View Software main screen.

ANALYZING THE IMAGE

A free full version of the Quansys Q-View™ Software is available to demo for 20 days. The download is available at:

www.quansysbio.com/support/softwareFiles.

User name: cytokine

Password: 8Y79m71W

Following the demo period the software will continue to allow the user to retrieve raw pixel intensity values from the imaged Q-Plex Array. This data can then be imported into another data analysis software package.

After the 20 day demo period a full license can be purchased by calling Quansys at 1-888-782-6797.

b. Click on "File," then select "Versadoc."

c. Make sure that only "Channel 1" is enabled.

d. Click on the "Select" button, then select "Custom" and then "Create."

e. When the new window opens, name this custom setup as "Quansys 1X1 binning," and change the settings to:

i. Filter: None

ii. Illumination: None

iii. Gain: 1X

iv. Binning: 1X1

f. Click on "OK." (Now that this setting is saved you can use it again for future exposures. Instead of selecting "create," select "Quansys 1X1 binning.")

2) Camera Setup & Focus

a. Open the imager door.

b. Place a box or stand in the cabinet below the camera to increase the imaging height between 4 and 6 inches.

c. Place the "imaging target sheet" or a mouse pad in the cabinet on top of the box or stand.

d. Open the aperture on the camera all the way to the lowest value.

e. Leave the door slightly open to let in light while focusing.

f. Click on "Focus" in the software, and turn the focus on the camera until the imaging targets on the screen on the computer are in focus. Click on "Stop" when in focus.

g. Replace the "imaging target sheet" with the plate, and ensure the plate is centered in the imaging screen and is straight.

- 10) Place the plate in the center of the tray and make sure it is in the center of the photo path (on the computer screen).
- 11) Close all doors on the camera and ensure there are no light leaks.
- 12) Make sure there are no lights turned on in the cabinet and that the filter wheel is set to "1."
- 13) Set the software settings on the computer.
 - a. No lights on.
 - b. Resolution to "Normal/Ultra."
 - c. Nothing should be checked in the boxes but "noise reduction."
 - d. Set the exposure time to 6min for the first time.
- 14) Click on **Acquire Image**.
- 15) Once the image is acquired, save it and look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000-55,000 Pixel Intensity range, and on the second spot they should be in the 20,000-40,000 pixel intensity range.
 - a. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then acquire another image of the plate for a shorter period of time.
 - b. If the spots on the high point of the curve in general fall below 40,000 PI, then reacquire the image for a longer period of time.

APPENDIX D: BIO-RAD VERSADOC 4000 CAMERA AND SOFTWARE SETUP

- 1) File Setup
 - a. Open the software on the computer.

APPENDIX A: INTERFERENCE AND COMPATIBILITY

Tissue culture supernatant, serum, plasma, urine, cell extracts, and tissue homogenates are compatible with the assay. Inhibitors are listed below:

| | |
|---------|-----------------------------|
| | |
| NP40 | No interference at 1% |
| Tween | No interference at 1% |
| Triton | No interference at 1% |
| Citrate | No interference at 20% |
| SDS | Interferes with Assay |
| EDTA | No interference at 20 mM |
| Water | No interference at 55 M |
| Heparin | No interference at 30 mg/ml |
| Urea | No interference at 1 M |
| DMEM | No interference at 100% |
| HAMS | No interference at 100% |
| RPMI | No interference at 100% |
| SFM4 | No interference at 100% |

APPENDIX B: ALPHA INNOTECH HD2 CAMERA AND SOFTWARE SETUP

- 1) Open the camera door.
- 2) Set the adjustable tray on the lowest level.
- 3) Place the mouse pad in the center of the tray for focusing.
- 4) Open the aperture on the camera all the way to the lowest value (~.95).
- 5) Open the software on the computer.
- 6) Click the Acquire button.
- 7) Close the door on the camera slightly so some light can get in and you can see the mouse pad on the computer screen.
- 8) Adjust the focus on the actual camera lens until the letters on the mouse pad are in focus.
- 9) Remove the mouse pad.
- 10) Place the plate in the center of the tray and make sure it is in the center of the photo path (on the computer screen).
- 11) Close all doors on the camera and ensure there are no light leaks.
- 12) Make sure there are no lights turned on in the cabinet and that the filter wheel is set to "1."
- 13) Set the software settings on the computer.
 - a. No lights on.
 - b. Resolution to "Normal/Ultra."
 - c. Nothing should be checked in the boxes but "noise reduction,"

d. Set the exposure time to 3min for the first time.

- 14) Click on [Acquire Image](#).
- 15) Once the image is acquired, save it and look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000-55,000 Pixel Intensity range, and on the second spot they should be in the 20,000-40,000 pixel intensity range.
 - a. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then acquire another image of the plate for a shorter period of time.
 - b. If the spots on the high point of the curve in general fall below 40,000 PI, then reacquire the image for a longer period of time.

APPENDIX C: ALPHA INNOTECH FC2 CAMERA AND SOFTWARE SETUP

- 1) Open the camera door.
- 2) Set the adjustable tray on the top shelf.
- 3) Place the mouse pad in the center of the tray for focusing/
- 4) Open the aperture on the camera all the way to the lowest value (~1.8).
- 5) Open the software on the computer.
- 6) Click the [Acquire](#) button.
- 7) Close the door on the camera slightly so some light can get in and you can see the mouse pad on the computer screen.
- 8) Adjust the focus on the actual camera lens until the letters on the mouse pad are in focus.
- 9) Remove the mouse pad.