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B I O S C I E N C E S

Quansys Q-Plex™ Array Mouse IR

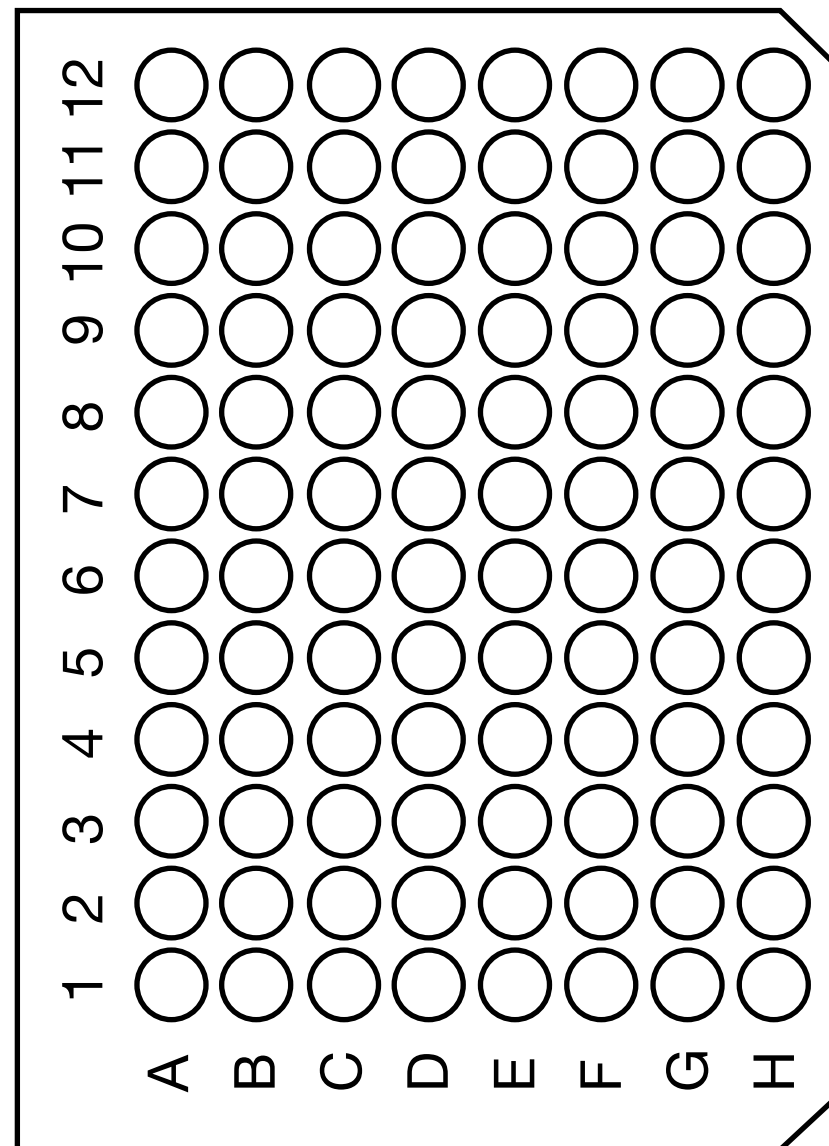
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ABBREVIATED PROTOCOL

- 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 diluent.
- 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 2 hours.
- 9) Wash the plate 3 times.
- 10) Add the detection mix to every used well on the plate.
- 11) Incubate the Detection mix for 2 hours.
- 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.

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.

SETUP OF LI-COR[®] ODYSSEY[®] OR AERIUS[®]

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	9-12
Origin	X=0, Y=0	N/A
Size	Width=13, Height=9	N/A

KIT CONTENTS

- 1) 96-well plate — multiplexed array bound and blocked in each well of the plate
- 2) Antigen standard — lyophilized
 - 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
- 4) IRDye® 800CW Streptavidin – lyophilized (50 µg)
- 5) IRDye® 800CW Dilution Buffer
- 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)

APPENDIX A: INTERFERENCES AND COMPATIBILITY

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

Inhibitor	Interference
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%

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.

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.

REQUIRED SUPPLIES

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

- 1) LI-COR® Odyssey® or Aeries® Infrared Imaging System
- 2) 8- or 12-channel pipette (20-200 µl) and/or 1-channel pipette and tips
- 3) Plate shaker
- 4) Paper towels
- 5) Kimwipes® (or equivalent)
- 6) 70% ethanol
- 7) Deionized water

VIDEO MANUAL

- 1) A video demonstration on each step of running the assay is available to download at www.quansysbio.com/support/manuals.

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. If lyophilized reconstitute using Sample Diluent according to the Antigen Standard card specifications.
- b. Mix gently until fully reconstituted.
- c. Store unused antigen standard at -20°C. Good for 1 week.

3) Detection antibody mix

- a. If lyophilized 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) IRDye® 800CW Streptavidin

- a. Add 50 µl of deionized water.
- b. Mix gently until fully reconstituted.
- c. Store unused IRDye® 800CW Streptavidin at 4°C. Good for 1 year.

Note: We recommend reconstituting the IRDye® 800CW Streptavidin 24-hours before running the test.

5) IRDye® 800CW

- c. Add 4 µl IRDye® 800CW Streptavidin to the IRDye® 800CW Dilution Buffer.
- d. Mix gently to ensure sufficient mixing.

Note: Do not allow IRDye® 800CW Streptavidin to be exposed to light for extended periods of time.

9) Rinse the plate

- a. Rinse the wells by hand with 100-200 µl of deionized water.
- b. Decant the wells immediately.

10) Dry the plate

There are two methods to dry the plate before imaging.

- a. Centrifuge – Place the plate face down on a dry paper towel. Centrifuge the plate at 300G for 2 minutes.
- b. Air Dry – Vigorously tap the plate upside down on a papertowel to remove excess water. Place the plate in open air and allow to dry for 10 minutes at room temperature.

11) Clean the plate

- a. Using Kimwipes® and 70% ethanol, clean the bottom of the plate and the surface of the scanner to help eliminate background caused by dust.

12) LI-COR® Odyssey® or Aeries® scanning of the plate

- a. Make sure settings are correct.
- b. Perform an initial scan.
- c. Adjust the intensity up or down depending on the brightness of each analyte.

Note: The optimal intensity assures that the majority of the systems high points in the standard curve approach saturation (65,000 pixel intensity) without saturation of any second dilution points.

- d. Multiple scans of varying intensities are recommended. It may be necessary to analyze multiple scans to obtain the best results for each analyte.

3) Add samples to the 96-well plate

- a. Add 30 μ l of sample and standard to each well. (See diagram on page 19 to plan the location of samples and standard. It is recommended to place the standard curve in the top left hand side of the plate running horizontally. This will allow for easier placement of grids when analyzing plates.)
- b. Cover the plate and place on a plate shaker for 2 hours at room temperature.

Note: 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 2 hours at room temperature.

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

7) Add IRDye® 800CW Streptavidin

- a. Add 30 μ l of the IRDye® 800CW Streptavidin 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).

6) 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.

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.
- 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.

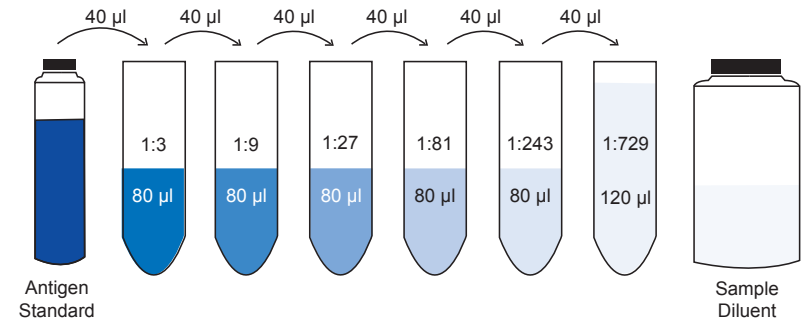
Multichannel Pipette Method

- 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 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 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 80 μl 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 human samples must be diluted with sample diluent to prevent interference from human serum.
 - 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.