

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

Q -Plex™ Array Human IR

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IMPORTANT PRECAUTIONS

1. **Read all instructions before beginning test.**
2. For research use only. Not for use in diagnostic procedures.
3. The kit should not be used beyond the expiration date on the kit label.
4. Do not mix or substitute reagents with those from other kits or lots.
5. 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

Q-VIEW™ SETUP

A free full version of the Quansys Q-View™ Software is available to demonstrate for 20 days.

Send a request for a free copy to info@quansysbio.com.

Following the demonstration 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 demonstration period, a full license can be purchased by calling Quansys at 1-888-782-6797.

KIT CONTENTS

1. 96-well plate —array spotted and blocked
2. Antigen standard — lyophilized
3. Detection mix
4. DyLight® IR Dye* — lyophilized
5. IR Dye Dilution Buffer
6. Sample diluent
7. Wash buffer 20X
8. Plate seals (2)

*DyLight® is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.

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 (20-200 µl) and tips
3. Paper towels
4. Kimwipes® (or equivalent)
5. Deionized water
6. Plate shaker
 - a. Platform Shaker or Microtiter Plate Shaker
 - b. Plate shaking speed is very important to assay performance.** Please see Appendix B for more specific plate shaker set up instructions.

VIDEO MANUAL

1. A video demonstration on each step of running the assay is available to download at www.quansysbio.com/support/files/videos
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 until kit expiration date.

2. Antigen standard

- a. Reconstitute lyophilized antigen standard using Sample Diluent according to the **Antigen Standard card** specifications. (The Antigen Standard card is accompanying the kit.)
- b. Mix gently until fully reconstituted.
- c. Store unused antigen standard at -20°C. Good for 1 week.

3. Detection antibody mix

- a. This solution comes mixed and ready for use.
- b. Store unused detection mix at 4°C. Good until kit expiration date.

4. DyLight® IR Dye

- a. Add 100µl of IR Dye Dilution Buffer to DyLight® IR Dye.
- b. Mix gently until fully reconstituted.
- c. Add 100µl of reconstituted DyLight® IR Dye back into remaining IR Dye Dilution Buffer.
- d. Mix gently.
- e. Store unused DyLight® IR Dye at 4°C. Good until kit expiration date.

Note: DyLight® IR Dye is very sensitive to light. Do not expose to direct light.

5. Wash buffer

- a. Place 50 ml of the 20X wash buffer into 950 ml deionized water.
- b. Invert bottle to ensure sufficient mixing.
- c. Store unused wash buffer at 4°C. Good until kit expiration date.

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. Ensure 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 shake 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.
7. Ensure washer tips do not touch the bottom of the 96-well plate.

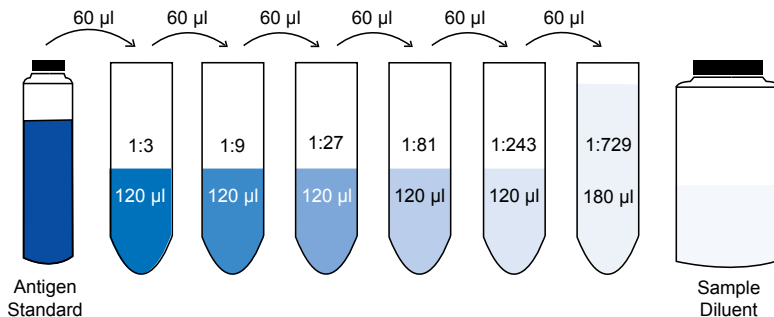
Multichannel Pipette Method

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

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. Doing so will validate the accuracy of the standard from one platform to another.

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



1. Prepare an 8-point standard curve (7 points plus 1 blank).
 - a. Pipette 120 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 serum/plasma samples **must** be diluted at least 1:2 (50%) 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. (See Appendix A.)

3. Add samples to the 96-well plate
 - a. Add 50 μ l of sample and standard to each well. (See diagram on page 18 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.*
 - *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 non-binding 96-well v-bottom plate and transfer it when ready.*
 - b. Cover the plate and place on a plate shaker for one hour at room temperature.
 4. Wash the plate three times using one of the methods described previously (see page 5).
 5. Add Detection Mix
 - a. Add 50 μ l of the Detection Mix to each of the wells.
 - b. Cover the plate and place on a plate shaker for one hour at room temperature.
 6. Wash the plate three times using one of the methods described previously (see page 5).
 7. Add DyLight® IR Dye
 - a. Add 50 μ l of the DyLight® IR Dye to each of the wells.
 - b. Cover the plate and place on a plate shaker for 15 minutes at room temperature.
- Note:** DyLight® IR Dye is sensitive to direct light. Make sure to minimize the amount of time the dye is exposed to light.
8. Wash the plate six times using one of the methods described previously (see page 5).

9. Rinse the plate

- a. Rinse the plate by hand with 100-200µl 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.

Note: DyLight® IR Dye is sensitive to direct light. The plate may be scanned up to 24 hours after running the test if kept in the foil bag at 4°C.

IMPORTING AN IMAGE FILE

To import an image taken with an imaging system other than the Q-View Imager, 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**.

Q-View Software can process images in the following formats: TIFF, JPEG, PNG and BMP.

After the image has been imported, it will appear in the Q-View Software main screen.

ANALYZING THE IMAGE

For image analysis instructions, see the Q-View Software Manual.

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:

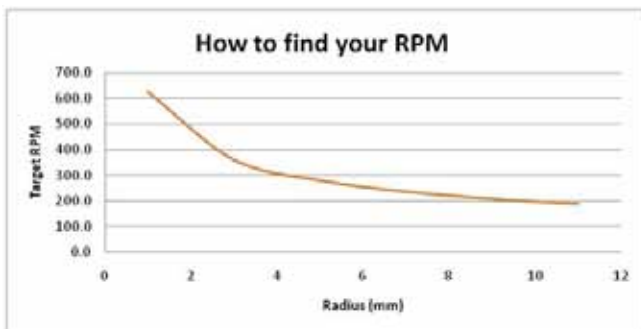
NP40	No interference at 1%
Tween	No interference at 1%
Triton	No interference at 1%
Citrate	No interference at 20%
SDS	Interferes with Assay
EDTA	No interference at 20 mM
Water	No interference at 55 M
Heparin	No interference at 30 mg/ml
Urea	No interference at 1 M
DMEM	No interference at 100%
HAMS	No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

Disclaimer: All products are carefully tested, however due to the variability encountered in biological buffers and sample matrices, the possibility of interference cannot be excluded.

APPENDIX B: PLATE SHAKING

To achieve the best results with the Q-Plex technology, consider calibrating your shaker.

1. Determining the Radius
 - a. To measure the radius, fasten a marker to the side of your shaker, set a blank paper below, and allow the pen to scribe the rotation path of the shaker. Measure the rotation path in millimeters. This radius can then be used with the following graph to determine the optimal RPM setting for your shaker.



2. Calibrating the Shaker
 - a. It is possible that your shaker's RPM speed dial directly correlates to the actual RPM of the shaker platform but this is typically not the case.
 - b. To manually determine the shaker's RPM, fasten a marker to the side of your shaker, and set a timer for 5 seconds. Once the timer begins, elevate a sheet of paper until the marker makes contact with the paper. Pull the paper horizontally for the 5 second interval and drop the paper once the alarm sounds. Once completed, count the number of oscillations and multiply by 12 to calculate the RPM at that setting. Adjust the shaker setting according to the optimum RPM setting you calculated in the first step.
 - c. It is recommended that you repeat this procedure at least 3 times to ensure accuracy and precision.

- d. After the shaker is calibrated, but prior to shaking a Q-Plex array, use a microtiter plate with buffer in it to make sure that the liquid is not splashing up and out of the wells. It should also be covering the center of each well while shaking.
- e. For a video demonstration on calibrating your shaker please visit [www.quansysbio.com/support files/videos](http://www.quansysbio.com/support/files/videos)

NOTES

NOTES

ABBREVIATED PROTOCOL

1. Set up the LI-COR® Odyssey® or Aerius® Infrared Imaging System. (page 2)
2. Determine the method of plate washing and prepare the wash buffer. (page 6)
3. Reconstitute the lyophilized vials by adding the indicated amount of diluent. (page 4) Also see Antigen Standard card insert for more information.
4. Prepare the standard curve by doing 1:3 serial dilutions of the Antigen Standard with the Sample Diluent. (page 8)
5. Prepare the samples. (page 8)
6. Add the standard curve to the top left hand side of the plate running horizontally. (page 9)
7. Add the samples to the plate. (page 9)
8. Incubate the samples and standard curve for 1 hour, with shaking. (page 9)
9. Wash the plate 3 times. (page 9)
10. Add the Detection Mix to every used well on the plate. (page 9)
11. Incubate the Detection Mix for 1 hour. (page 9)
12. Wash the plate 3 times. (page 9)
13. Add the DyLight® IR Dye to every used well on the plate. (page 9)
14. Incubate the DyLight® IR Dye for 15 minutes. (page 9)
15. Wash the plate 6 times. (page 9)
16. Hand rinse the plate with deionized water, then decant. (page 9)
17. Dry the plate. (page 10)
18. Clean the bottom surface of the plate and the scanner surface. (page 10)
19. Scan the plate on LI-COR® Odyssey® or Aerius® Infrared Imaging System.(page 10)

PLATE DIAGRAM

1	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
3	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
4	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
6	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
7	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
8	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
9	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
10	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
11	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
12	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
	A	B	C	D	E	F	G	H									