QUANSYS BIOSCIENCES

Q-Plex™ ARRAY

Chemiluminescent Training Kit

For Research Use Only Version 2.1
Not For Use In Diagnostic Procedures

QUANSYS BIOSCIENCES

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Symbol	Explanation
REF	Catalog Number
LOT	Lot Number
	Use By YYYY-MM-DD
	Temperature Limitation
	Manufacturer
	Keep Away From Sunlight


Manufactured and Distributed by:
Quansys Biosciences
365 North 600 West Logan, Utah 84321, USA
TEL: (888) 782-6797
E-MAIL: INFO@QUANSYSBIO.COM

For International Distributors see:
http://www.quansysbio.com/distributors

NAME AND INTENDED USE

Q-Plex™ Chemiluminescent Training Kit
Quansys Biosciences Catalog Number 100749GR
Software product code: CALIBRATION

The Q-Plex Chemiluminescent Training Kit is used to train and educate on how to use the Q-View Imager and Software. Each well of this kit contains 9 spots, all producing the same signal.

PRINCIPLE OF THE ASSAY

This multiplex assay is not an ELISA, but a simple kit to quickly produce spots for the use of training and education.

These assays use a biotinylated protein in an array format on the bottom of the well. The user then adds streptavidin-horseradish peroxidase (SHRP) and incubates for 15 minutes. A wash of the plate will then remove any residual SHRP. Chemiluminescent substrate is added and the plate is then imaged. Any SHRP still bound will emit a signal which is digitally recorded by the Q-View Imager.

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.

DO

- Do set up and practice using the Q-View™ Imager Pro or Q-View™ Imager LS before starting the assay.
- Do be exact when setting shaker speed to 500 RPM.
- Pre-wet pipette tips three times by drawing up the liquid into the pipette and then dispensing back into the original vessel prior to the addition of samples or calibrators to the microplate.
- Do be exact with incubation times, particularly the streptavidin-horseradish peroxidase (SHRP) incubation.
- Do be exact when mixing Substrate A and B+ and mix thoroughly.

DO NOT

- Do not allow the plate to dry out between steps.
- Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade it.

KIT CONTENTS & STORAGE

Unopened Kit -  Store at 2-8°C. Do not use past kit expiration date. Do not re-use the kit.

Part/Description	Storage of opened/reconstituted material
Q-Plex™ Array Microplate Arrayed and blocked 96-well polystyrene microtiter plate	 2-8°C until kit expiration
Wash Buffer Concentrate (20X) Liquid, 50 mL/vial of a concentrated solution of buffered surfactant	
Streptavidin-HRP 1X Liquid, 6 mL/vial of streptavidin-conjugated horseradish peroxidase	 Do not expose to UV light. 2-8°C until kit expiration
Substrate A Liquid, 3 mL/vial of stabilized hydrogen peroxide	 Do not expose to UV light. Store mixed substrate solution at room temperature (20-25°C) for up to 1 week. Store unmixed solutions at 2-8°C until kit expiration.
Substrate B+ Liquid, 3 mL/vial of stabilized signal enhancer	
Plate Seals (2) Adhesive strips	Non-perishable

OTHER REQUIRED MATERIALS: INSTRUMENTS AND ACCESSORIES

In addition to the kit contents listed, the following materials are required to run this assay.

1. Multichannel pipette (20-200 µL) and/or a single channel pipette (20-200 µL) and tips
2. Polypropylene tubes or polypropylene 96-well plate(s) for sample and calibrator preparation
    a. Recommended: Nunc® MicroWell™ 96-Well Plates, Polypropylene, 249944; Eppendorf® LoBind Protein or Genomic Microcentrifuge Tubes, 022431102
3. Q-View™ Imager and Software
4. Microplate shaker
    a. For example: Barnstead/labline 4625 titer plate shaker, IKA MTS 2/4 for 2 or 4 microtiter plates, or equivalent, capable of 500 RPM.
5. Deionized water
6. Microplate washer
7. Graduated cylinder for the preparation of wash buffer

ASSAY PREPARATION

1. Install Q-View™ Software on the computers that will be used for analysis or operating a Q-View Imager Pro or Q-View Imager LS.
2. Set up the imager. For imager-specific instructions, see www.quansysbio.com/manuals.
3. Set the plate shaker to 500 RPM.
4. Prepare the Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water, and mix thoroughly.
5. Allow Substrate A and B+ to come to room temperature (20-25°C). Fifteen minutes prior to use, combine 3 mL of Substrate A with 3 mL of Substrate B+, and mix gently. **Do not expose to UV light. Store at room temperature (20-25°C) after mixing.**

ASSAY PROCEDURE

Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed by the previous sections.

1. Wash the plate three times.
2. Add 50 µL per well of Streptavidin-HRP 1X, cover with a new plate seal, and return to the plate shaker set to 500 RPM for 15 minutes at room temperature (20-25°C).
3. Wash the plate six times (see Appendix A).
4. Add 50µL per well of previously prepared substrate. Wait no longer than 15 minutes to commence imaging.

   Note: If imaging cannot commence immediately, protect the plate from drying for up to 15 minutes by dispensing 100 µL of wash buffer into each well of the plate prior to adding the mixed substrate. When ready to image, remove the wash buffer from the plate and add the substrate.
5. Place the plate in the Q-View Imager Pro or Q-View Imager LS.
6. Open Q-View Software, create or open a project, and click Acquire Image.

7. When using a Q-View Imager Pro, set the exposure time to 300 seconds.
8. When using a Q-View Imager LS, set the exposure time to 270 seconds and standard image processing.
9. Click the Capture Image(s) button. Users may continue on to Well Assignment while images are being captured.

Details about these imaging steps are available in the Q-View Software Manual viewable at www.quansysbio.com/manuals or within Q-View Software under **Support > Manual**.

ANALYZING A Q-PLEX™ IMAGE

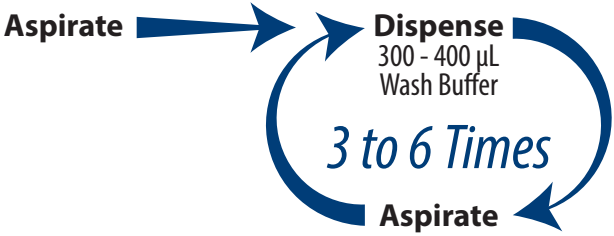
The following summarizes a general workflow for analyzing a Q-Plex image in Q-View Software. Each of these steps is described in greater detail in the Q-View Imager and Software Manual, viewable at www.quansysbio.com/manuals, or within Q-View Software under **Support > Manual**.

1. Acquire or import an image into Q-View Software as described above.
2. Enter the product code, **CALIBRATION** into the **Product Code** field.
3. **Image Processing**: Align the plate overlay as follows:
    a. To visualize bright or dim spots, optimize the display using **Image Options > Adjust Gamma** (does not affect the data).
    b. Set the overlay: If using the **Auto-Set Plate Overlay** feature, this will occur automatically. Otherwise, go to **Overlay Options > Set Plate Overlay.**
    c. Optimize overlay alignment: Go to **Overlay Options > Adjust Plate Overlay** to pivot the overlay, **Adjust Well** and **Adjust Spot** to move individual wells and spots, then **Auto-Adjust Spots** to automatically snap each circle of the overlay to the nearest spot of the image beneath.
    d. **Well Assignment:** Label wells as samples, controls, calibrators, or negatives, and specify their dilution factors. Use **Templates** to quickly assign layouts that are repeated often, or export the layout as a .csv file.
4. **Data Analysis:** Once you have completed **Image Processing** and **Well Assignment,** select **Data Analysis**. Click **Perform Analysis** to generate charts, concentrations, and statistics based on optimal regression settings provided by the product definition.

Tips for data analysis are available at www.quansysbio.com/tech-tips. We take great care to ensure that customers have success using our products and services. If you have further questions about running the assay, data analysis, troubleshooting, or our products or services, please contact us at 888-QUANSYS (782-6797) or at SUPPORT@QUANSYSBIO.COM

APPENDIX A: PLATE WASHING METHOD

1. Use a program that will aspirate and dispense 300-400 µL wash buffer.



2. Ensure that the plate washer has a program that will not scratch the bottom of the microplate and will prevent plate drying. This is critical to prevent damage to the capture antibody arrays. The simplest method to avoid plate drying is to leave a small, uniform amount (1-3 µL) of wash in the well after the final aspiration, and add the next reagent to the plate as quickly as possible.

3. Leaving a uniform amount of wash in the wells can be accomplished with an automated washer by slightly increasing the aspiration height of the washer head. *For example:*

Process	**Distance**	**Steps on a Biotek ELX-405**
Aspiration Height	3.810 mm	30
Aspiration Position	1.28 mm from center	-28
Dispense Height	15.24 mm	120
no soak or shake cycles are needed		

4. Connect the prepared wash buffer to your automatic plate washer.
5. 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.
6. To ensure that all pins are functioning, in a spare microtiter plate, dispense 100µL wash buffer and ensure that all pins dispense uniformly, then aspirate and ensure that all pins aspirate uniformly.
7. Prime the plate washer one time before the first wash step. When running the assay, perform the wash 3 or 6 times according to the protocol.

ABBREVIATED PROTOCOL

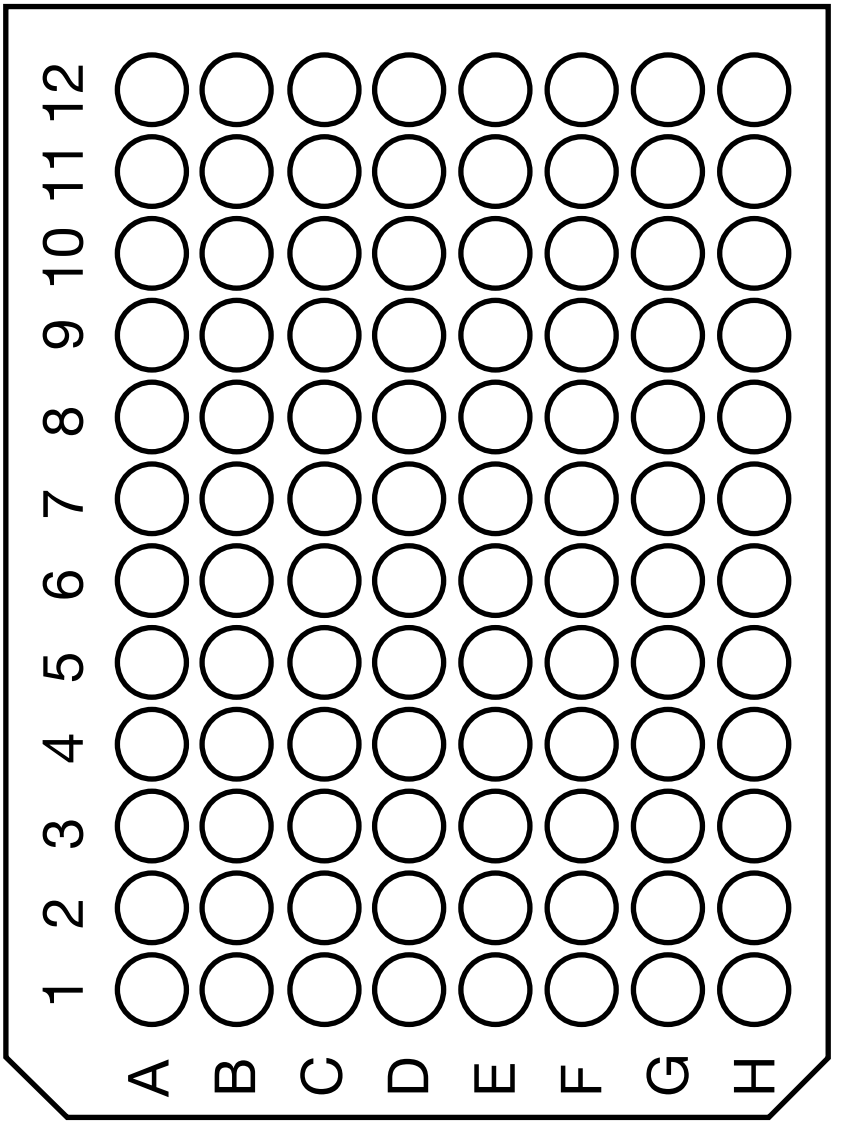
Preparation

1. Install Q-View Software *(page 6)*.
2. Set up the imager *(page 6)*.
3. Set up microplate washer *(page 9)* and shaker *(page 6).*
4. Reconstitute and prepare reagents *(page 5).*

Running the Assay

5. Wash the plate three times, add the Streptavidin HRP 1X, shake for 15 minutes at room temperature, (500 RPM) *(page 9)*.
6. Allow Substrate A and Substrate B+ to come to room temperature, then mix equal volumes and allow the solution to sit at room temperature *(page 6)*.
7. Wash the plate six times, and add the mixed Substrate *(page 6)*.
8. Capture and analyze image of the plate *(page 6)*.

PLATE DIAGRAM



www.quansysbio.com

365 North 600 West, Logan, Utah 84321

T: 1-888-782-6797 • F: (435)750-6869

www.quansysbio.com • Technical Support: support@quansysbio.com

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