QUANSYS BIOSCIENCES

Q-Plex™ ARRAY
Chemiluminescent

For Research Use Only Version 2.5

QUANSYS BIOSCIENCES

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Manufactured and Distributed by:
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For International Distributors see:
http://www.quansysbio.com/distributors

IMPORTANT PRECAUTIONS

1. **Read all instructions before beginning test.**
2. **For research use only.**
3. The kit should not be used beyond the expiration date on the kit label. Send requests for replacement reagents to: INFO@QUANSYSBIO.COM.
4. This kit is susceptible to saliva contamination. We recommend wearing a mask during preparation and running of kits.
5. If running multiple kits, a standard curve must be included for each 96-well plate. The curve from one plate cannot be used to calculate sample values from other plates.
6. Do not mix or substitute reagents with those from other kits or lots.
7. This kit is validated for use with plasma, serum, and cell culture supernates. If samples generate analyte values higher than the highest standard, further dilute the samples with sample diluent and repeat the assay.
8. When comparing results from this kit to other platforms, test the same antigen standards and samples on both platforms. Doing so will validate the accuracy of the standard from one platform to another.
9. All products are carefully validated, however due to the variability encountered in biological buffers and sample matrices, the possibility of interference or sample matrix effects cannot be excluded.

Q-VIEW™ SOFTWARE

A free copy of the Q-View Software, a tool for the quantitative analysis of multiplex ELISAs and for controlling Q-View Imagers, is available to all Q-Plex users. Please send requests to download the software to INFO@QUANSYSBIO.COM.

A summary of how to use the Q-View Software to analyze an image is contained in this manual (Page 9). The full Q-View Software Manual is also available at www.quansysbio.com/manuals.

DO'S

- DO set up, calibrate, and practice using the Q-View imager BEFORE starting the assay.
- DO be exact when setting shaker speed to 500 RPM, being off by even 100 RPM can affect results.
- DO dilute all sample types at least 1:2 (one part sample to one part diluent) with the provided sample diluent (except cell culture samples, which can be tested NEAT) to prevent false positives, and mix thoroughly.
- DO load all standards and samples into the microplate within 10 minutes of each other.
- DO be exact with incubation times, particularly the SHRP incubation.
- DO be exact when mixing Substrate A and B, being off by even 100 µL can affect results, and mix thoroughly.

DON'TS

- DON'T allow the plate to dry out between steps.
- DON'T allow the substrate or SHRP to be exposed to UV light, as this may degrade it.
- DON'T analyze from a jpeg, bmp, or png image; only TIFF or other full resolution, lossless file types with at least 16-bit depth are acceptable for analysis.

Further tips on troubleshooting, data analysis, and assay sensitivity 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 any further questions about the assay or our products or services, please contact us at 888-QUANSYS (782-6797) or at INFO@QUANSYSBIO.COM.

IMAGING SYSTEMS

1. Recommended imager optimized for use with Q-Plex Arrays:

   Quansys Q-View™ Imager (Cat #104450GR) with Q-View™ Software

2. Suggested imagers that are commonly used with Q-Plex™ Arrays:

   Bio-Rad: Versa Doc 4000 or Chemi Doc XRS
   GE Healthcare (formerly Fujifilm): (with NP Tray accessory)LAS-3000, LAS-3000 Mini, LAS-4000
   LI-COR: Aerius®, Odyssey-CLx®

3. Other imagers that may be compatible, for use with Q-Plex™ Arrays:

   Alpha Innotech: Fluorchem HD, SP, 8000, 8900, 9900, HD2, and FC2
   Fujifilm: LAS-4000 Mini
   Carestream (formerly Kodak): 4000MM, 2000MM, Gel Logic 100
   UVP: BioDoc-IT System, EC3 Darkroom

For imager-specific imaging instructions, see www.quansysbio.com/compatible-imagers.

For minimum imager requirements, see www.quansysbio.com/third-party-imagers.

To receive a FREE Calibration Kit that can be used to set up and validate an imager, contact us at 888-QUANSYS (782-6797) or at INFO@QUANSYSBIO.COM.

KIT CONTENTS, PREPARATION, & STORAGE

Part	Description	Reagent Preparation	Storage of opened/ reconstituted material*
Q-Plex Array Microplate	Spotted and blocked 96-well polystyrene microtiter plate	Ready for use	If the plate contains unused wells, return the plate to the sealed foil pouch at 4°C
Wash Buffer Concentrate (20X)	Liquid, 50 mL/vial of a concentrated solution of buffered surfactant	Place 50 mL of the 20X concentrate into 950 mL deionized water, mix thoroughly.	4°C until kit expiration
Antigen Standard	Lyophilized, recombinant antigens in a buffered protein base See the Antigen Standard Card for high points.	When ready to begin the assay, reconstitute in Sample Diluent according to the Antigen Standard Card which accompanies the kit. Mix gently until fully reconstituted.	Discard unused reconstituted antigen. Good for one day.
Sample Diluent	Liquid, 10 mL/vial of a buffered protein solution with heterophilic antibody and rheumatoid factor blockers, and preservatives	Ready for use	4°C until kit expiration
Detection Mix	Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives	Ready for use	
Streptavidin-HRP 1X	Liquid, 6 mL/vial of streptavidin-conjugated horse radish peroxidase	Ready for use. Do not expose to UV light.	Do not expose to UV light. 4°C until kit expiration
Substrate A	Liquid, 3 mL/vial of stabilized hydrogen peroxide	Do not expose to UV light. Do not cool after mixing. During the assay, mix 3 mL of Substrate A with 3 mL of Substrate B, and mix gently.	Do not expose to UV light. Do not cool after mixing. Store mixed substrate solution at room temperature for up to 1 week. Store unmixed solution at 4°C until kit expiration.
Substrate B	Liquid, 3 mL/vial of stabilized signal enhancer		
Plate Seals (3)	Adhesive strips	None	Non-perishable
**Provided this is within the expiration date of the kit*			

OTHER REQUIRED MATERIALS: INSTRUMENTS AND ACCESSORIES

In addition to the kit contents listed, the following materials are required to run Q-Plex Assays or panels for optimal results. We reccommend the use of these specific items.

1. 8- or 12-channel pipette (20-200 µL) and/or 1-channel pipette (20-200 µL) and tips
2. Low-binding polypropylene tubes or low-binding 96-well plate(s).
3. Imaging system
4. Q-View™ Software
5. Microplate shaker
    a. Barnstead/labline 4625 titer plateshaker, IKA MTS 2/4 for 2 or 4 microtiter plates, or equivalent, capable of 500-1,100 RPM.
6. Optional: spare microtiter plate to test an automatic plate washer *(See Appendix B Step 4)*.

VIDEO MANUAL

A video tutorial on each step of the assay is available at www.quansysbio.com/videos-tutorials.

If a high-speed Internet connection is not available, contact us at 1-888-782-6797 for a free copy.

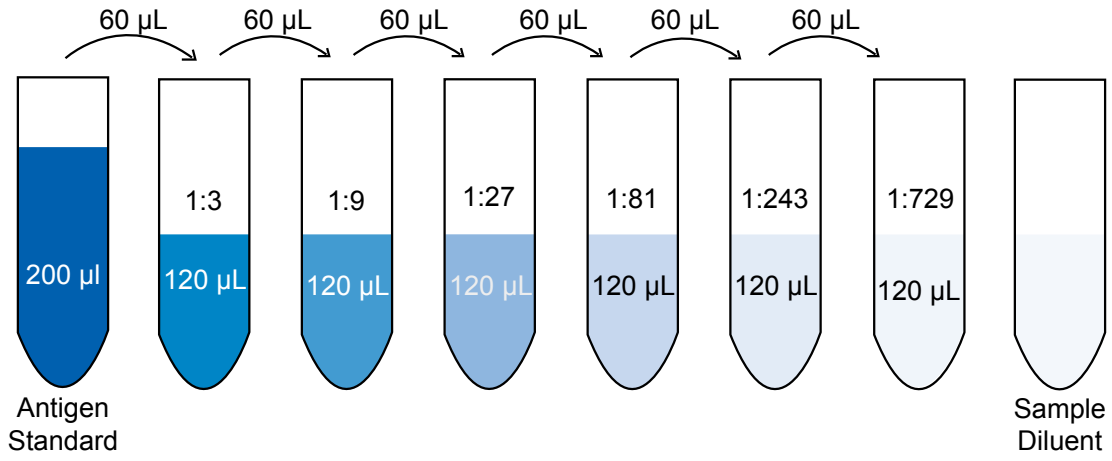
ASSAY PREPARATION

1. Install the FREE Q-View Software on any number of computers to be used for analysis or driving a Q-View Imager *(Page 4)*.
2. Set up the imager *(Page 9)*. For imager-specific instructions, see www.quansysbio.com/compatible-imagers. To receive a free Calibration Kit that can be used to validate imager settings, contact us at 888-QUANSYS (782-6797) or at INFO@QUANSYSBIO.COM.
3. Set the plate shaker to 500 RPM.
4. Choose a plate washing method *(Appendix B)*.
5. Prepare the Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water, and mix thoroughly. Store at 4˚C.

ASSAY PROCEDURE

Bring all reagents to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Reconstitute the Antigen Standard in Sample Diluent with the volume on the Antigen Standard Card which accompanies the kit, mix gently until fully reconstituted.
2. Prepare an 8-point standard curve (7 points plus 1 blank) in either low-binding polypropylene tubes or a low-binding 96-well plate.



a. Pipette 200 µL of the antigen standard into the first tube or well.

b. Place 120 µL of sample diluent into the other seven tubes or wells.

c. Transfer 60 µL of the undiluted antigen standard from the first tube into the second, mix thoroughly, and repeat the transfer from tube to tube for 5 more points, leaving the last tube without any antigen. This process is diagrammed above. The undiluted antigen standard serves as the high point of the standard curve. The sample diluent serves as the negative control.

3. Prepare serum and plasma samples by diluting at least 1:2 (one part sample to one part diluent) with enough sample diluent to have 50 µL per well in either low-binding polypropylene tubes or a low-binding 96-well plate.

   Note: Using alternate diluents may increase the chance of interference and false positives. If you anticipate that your analyte concentration will be higher than the ranges on the standard curves, use the sample diluent to dilute your samples further.

4. Add 50 µL per well of the antigen and samples to the Q-Plex Array 96-well plate. Load all samples and standards to the plate within ten minutes.

5. Cover the plate with the plate seal provided, and place on a plate shaker set to 500 RPM for one hour at room temperature (23°C).

   *Note: Record the plate layout in the Well Assignment section of the Q-View Software, in the Q-View compatible Excel template (**Well Assignment > Templates > New Template**), or using the plate diagram* *(See page 18).*

6. Wash the plate three times according to the preferred washing method *(see Appendix B)*.

7. Add 50 µL per well of Detection Mix, cover with a new seal, and return to the plate shaker set to 500 RPM for one hour at room temperature.

8. Wash the plate three times as in step 6.
9. Add 50 µL per well of Streptavidin-HRP 1X, cover with a new seal, and return to the plate shaker set to 500 RPM for 15 minutes at room temperature.
10. Allow Substrate A and B to come to room temperature. 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 after mixing.**
11. Wash the plate six times as in step 6.
12. Add 50µL per well of mixed substrate, and image the plate, as described below, immediately for optimal results. 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.

ACQUIRING AN IMAGE USING THE Q-VIEW™ IMAGER

1. Place the plate in the calibrated Q-View Imager and shut the lid.
2. Open the Q-View Software, create or open a project, and click Acquire Image.

   Recommended settings for Q-Plex™ kits are:

   F-Stop: 2.8

   ISO: 400

   Exposure Time(s)(seconds): 30, 60, 180

   Multiple exposure times can be set for image acquisition by typing the desired times, measured in seconds, separated by commas in the Exposure Time(s) field.

3. Click the Capture Image(s) button. Users can 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 the Q-View Software under **Support > Manual**.

ACQUIRING AN IMAGE FROM AN ALTERNATE IMAGER

Imager-specific imaging instructions for alternate imagers are available at www.quansysbio.com/compatible-imagers.

ANALYZING A Q-PLEX IMAGE

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

1. Acquire or import an image into Q-View as described above.

   *Note: Images with black spots on a white background MUST be inverted either by selecting the option during import, or by going to **Image Options > Invert** after importing. DO NOT analyze from a jpeg, bmp, or png image; only grayscale TIFF or other full resolution, lossless file types with at least 16-bit depth are acceptable for analysis.*

2. Enter the **Product Number** (found on the Antigen Standard Card) into the **Product** 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.**

c. Optimize overlay alignment: Go to **Overlay Options > Adjust plate** 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.

4. **Well Assignment**: Label wells as samples, controls, standards, 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.

5. **Data Analysis:** Choose a **Curve Fit Option**, mask outliers, and select limits. The software will automatically compile customizable reports with tables, charts, and statistical information. Finally, copy or **Export** the data as needed.

Further tips for data analysis are available at www.quansysbio.com/tech-tips. 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 INFO@QUANSYSBIO.COM

APPENDIX A: INTERFERENCE AND COMPATIBILITY

Plasma, serum, and cell culture supernates are validated for use with this assay. While not validated, the analyte levels of a wide variety of tissue homogenates and cell lysates, including mouse or human lung, liver, kidney, pancreas, kidney, eye, urine and brain, have been successfully measured using Q-Plex Arrays under standard conditions.

- For those testing such alternate sample types, we also offer:
- One FREE Demo Kit or FREE in-house sample testing of four samples.

Custom development: Quansys can perform optimization and validation of assay conditions specific for the user's sample type, or using custom antigens or antibodies.

Many common diluents/reagents used in collection and preparation of samples are compatible with Q-Plex Arrays. Use the reagent compatibility table below as a guideline in designing the optimal sample collection protocol for your sample type. For an example tissue homogenization protocol, see http://www.quansysbio.com/compatible-sample-types.

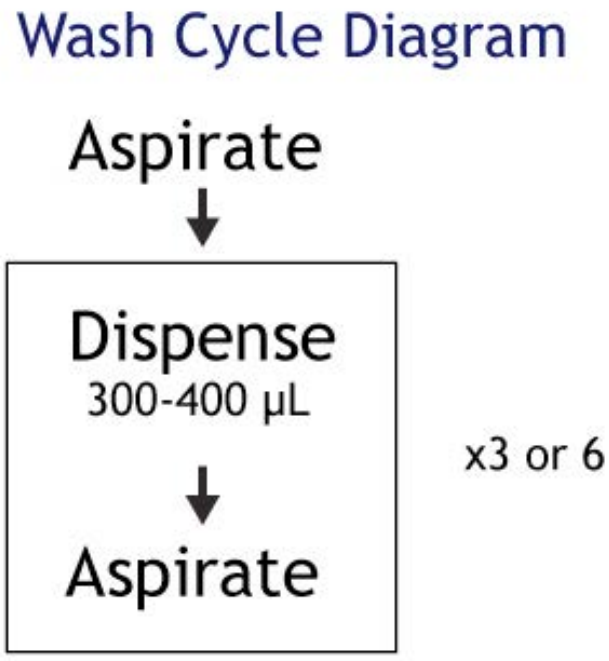
NP40	No interference at 1%
Tween	No interference at 1%
Triton	No interference at 1%
Citrate	No interference at 20%
SDS	Interferes with Assay
EDTA	No interference at 20 mM
Heparin	No interference at 30 mg/mL
Urea	No interference at 1 M
DMEM	No interference at 100%
HAMS	No interference at 100%
RPMI	No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

APPENDIX B: PLATE WASHING METHODS

Before running the assay, 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

Preferred: Automatic Plate Washer 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 spots. 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. For this reason, we do not recommend blotting the plate on a paper towel when using an automatic plate washer. 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		

3. Connect the prepared wash buffer to your automatic plate washer.
4. 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.
5. 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.
6. 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.

Multichannel Pipette Method

1. Just prior to washing, pour the prepared wash buffer into a trough or tray.
2. After each incubation, flick the solution out of the plate over a waste container before starting the wash protocol.
3. Using a multichannel pipette, dispense 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 assay procedure calls for three or six washes, repeat steps 3-4 accordingly.

ABBREVIATED PROTOCOL

Preparation

1. Install the FREE Q-View Software on any number of computers to be used for analysis or driving a Q-View Imager *(Page 4)*.
2. Set up the imager *(Page 4)*.
3. Determine the method of plate washing and prepare the wash buffer *(Page 13)*.

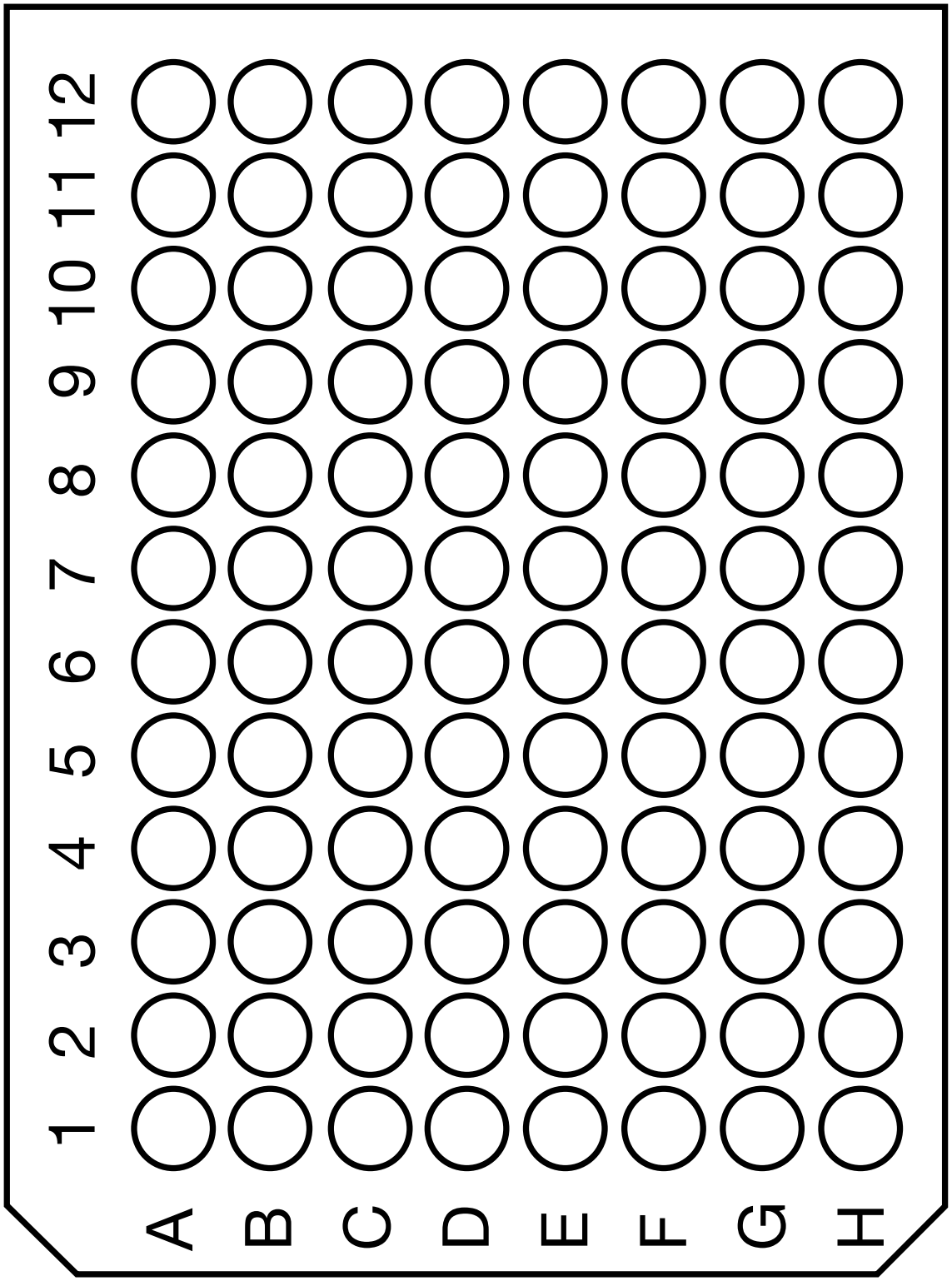
Running the Assay

4. Reconstitute the lyophilized vials by adding the indicated amount of diluent *(Page 5)*.
5. Prepare the standard curve with the Sample Diluent according to the Antigen Standard Card *(Page 7)*.
6. Prepare the samples with Sample Diluent *(Page 8)*.
7. Load the standard curve and samples onto the plate. Incubate for 1 hour at room temperature, with shaking at 500 RPM *(Page 8)*.
8. Wash the plate 3 times, add the Detection Mix, and incubate for 1 hour at room temperature, with shaking at 500 RPM *(Page 9)*.
9. Wash the plate 3 times, add the Streptavidin HRP 1X, incubate for 15 minutes at room temperature, with shaking at 500 RPM *(Page 8)*.
10. 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 8)*.
11. Wash the plate 6 times, and add the mixed Substrate *(Page 9)*.
12. Capture an image of the plate *(Page 9)*.

NOTES

NOTES

PLATE DIAGRAM



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