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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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QUANSYS BIOSCIENCES

Q-Plex™ ARRAY

Human Pneumococcal IgG (14-Plex)

For Research Use Only Version 1.1
Not For Use In Diagnostic Procedures

TABLE OF CONTENTS

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


Manufactured and Distributed by:
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http://www.quansysbio.com/distributors

NAME AND INTENDED USE

Q-Plex™ Human Pneumococcal IgG (14-Plex)
Quansys Biosciences Catalog Number 481749HU

The Q-Plex Human Pneumococcal IgG (14-Plex) is a quantitative chemiluminescent assay (ELISA) allowing concurrent measurement in serum and plasma samples of human IgG antibodies reactive to *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 12F, 14, 18C, 19F, and 23F polysaccharides. This kit is intended for research use only.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the indirect immunoassay technique for the measurement of human IgGs reactive to polysaccharides from 14 S. pneumoniae serotypes, *S. pneumoniae* cell wall polysaccharide (negative control) and human IgG (positive control).

The polysaccharides from 14 S. pneumoniae serotypes are arrayed in a microplate (1, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 12F, 14, 18C, 19F, 23F). The microplates are arrayed with positive and negative controls in each well.

Samples or calibrators are pipetted into wells of a 96 well plate arrayed with immobilized specific polysaccharides. After washing away any unbound IgG, a mixture that contains biotinylated anti-human IgG is added. After washing away unbound biotinylated antibody, streptavidin-horseradish peroxidase (SHRP) is added. Following an additional wash, the amount of SHRP bound to each location of the array is proportional to the amount of human IgG reactive to each of the specific polysaccharides, CWPS (negative control) and human IgG (positive control) initially captured.

The amount of conjugated enzyme on each location of the array is measured with the addition of a chemiluminescent substrate.

Assay Ranges

14-Plex	Units	Calibrator Range*	LLD
Serotype 1	µg/ml	9 - 0.012	0.008
Serotype 3	µg/ml	1 - 0.004	0.012
Serotype 4	µg/ml	3 - 0.004	0.009
Serotype 5	µg/ml	8 - 0.011	0.007
Serotype 6B	µg/ml	9 - 0.012	0.008
Serotype 7F	µg/ml	9 - 0.012	0.022
Serotype 8	µg/ml	14 - 0.019	0.114
Serotype 9N	µg/ml	7 - 0.010	0.019
Serotype 9V	µg/ml	7 - 0.010	0.017
Serotype 12F	µg/ml	2 - 0.025	0.018
Serotype 14	µg/ml	38 - 0.052	0.034
Serotype 18C	µg/ml	7 - 0.010	0.020
Serotype 19F	µg/ml	15 - 0.021	0.013
Serotype 23F	µg/ml	6 - 0.025	0.016

* Actual values may vary from kit to kit. Please see the antigen card included in your kit for specific values.

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions are intended as general guidelines. The Clinical and Laboratory Standards Institute (CLSI GP44-A4) recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C. If the assay will not be completed within 48 hours, or if the samples will be stored beyond 48 hours, samples should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed. Frozen samples must be mixed well after thawing and prior to testing. Diluted samples should be tested within 8 hours. Do not use bacterially contaminated samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine its own specific stability criteria.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, Citrate, or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

WARNINGS AND 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. **This kit is sensitive to saliva. Wear a mask during preparation and running of the kit.**
5. If running multiple kits, a calibration 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. Load all calibrators, controls, and samples into the microplate within 5 minutes of each other.
8. Be exact with incubation times, particularly the streptavidin-horseradish peroxidase (SHRP) incubation.
9. Do not allow the substrate or SHRP to be exposed to UV light, as this may degrade it.
10. Do not allow the plate to dry out between steps.
11. Be exact when mixing Substrate A and B+ and mix thoroughly.
12. Warning: The calibrator and controls contain components of human origin. These components have been tested at the donor level and found negative for HBsAg, HIV-1 and HIV-2 antibodies, and HCV. However, consider all materials as potentially infectious and use only approved guidelines for the proper handling and disposal of infectious material.

KIT CONTENTS, PREPARATION & 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
96-Well Q-Plex™ Plate 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	
Sample Diluent A 2X Liquid, 10 mL/vial of a buffered solution with blockers and preservatives at 2X concentration	
Detection Mix Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives	
Calibrator Lyophilized, recombinant antigens in a buffered protein base	 2-8°C until kit expiration Discard unused reconstituted calibrator.
Controls (High and Low) Lyophilized, recombinant antigens in a buffered protein base	 2-8°C until kit expiration Discard unused reconstituted Controls .
Streptavidin-HRP 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	 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	
Plate Seals (3) Adhesive strips	Non-perishable

MATERIALS REQUIRED BUT NOT SUPPLIED

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

1. Multichannel pipette (20-100 µL) and/or single channel pipettes (20-1000 µL) and tips
2. 10 mL serological pipette
3. 1 liter graduated cylinder for the preparation of wash buffer
4. Polypropylene tubes or polypropylene 96-well plate(s) for sample, calibrator and control preparation
    a. For example: Nunc® MicroWell™ 96-Well Plates, Polypropylene, 249944; Eppendorf® LoBindProtein or Genomic Microcentrifuge Tubes, 022431102
5. Q-view™ Imager and Software
6. Microplate washer (An optional multichannel pipette protocol is provided in Appendix A)
7. 50 mL conical tube for diluting the 2X Sample Diluent
8. Deionized water

ASSAY PREPARATION

1. Install Q-View Software™ on the computer that will be used for 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. Prepare Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water and mix thoroughly.
4. Prepare 1X Sample Diluent A: In a 50 mL conical tube add 10 mL of Sample Diluent A 2X and 10 mL of deionized water (20 mL total) and mix thoroughly.
5. Prepare Calibrator: Reconstitute using the 1X Sample Diluent A with the volume specified on the Product Card which accompanies the kit. Allow Calibrator to sit for at least 5 minutes. Mix thoroughly. Use Calibrator within 30 minutes of reconstitution.
6. Prepare Controls: Reconstitute using 1X Sample Diluent A with the volume specified on the label. Allow Controls to sit for 5 minutes. Mix thoroughly.
7. Allow Substrate A and B+ to come to room temperature (20-25°C). 15 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

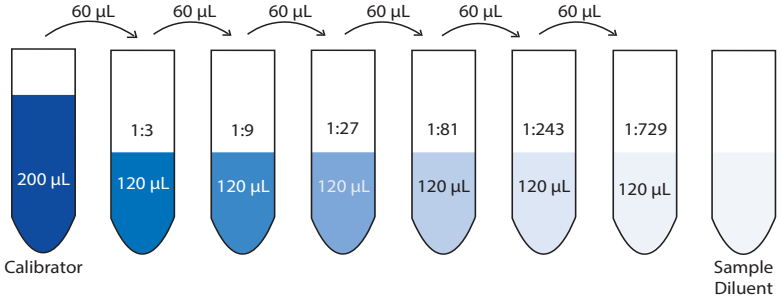
This assay is saliva sensitive. Wear a mask when running this kit. Allow all reagents to equilibrate to room temperature (20-25°C) before use and prepare as directed by the previous sections. It is recommended that all calibrators, samples, and controls be assayed in duplicate. This assay is optimized for no shaking.

1. Using the previously reconstituted calibrator, prepare an 8-point calibration curve (7 points plus 1 blank) in either polypropylene tubes or a polypropylene 96-well plate.

    a. Pipette 200 µL of the prepared calibrator into the first tube or well.

    b. Place 120 µL of prepared 1X Sample Diluent A into the other 7 tubes or wells.

    c. Transfer 60 µL of the undiluted prepared calibrator 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 prepared calibrator. This process is diagrammed below. The undiluted prepared calibrator serves as the high point of the standard curve. The prepared 1X Sample Diluent A serves as the negative.



2. Dilute samples 1:200 (1 part sample to 199 parts prepared 1X Sample Diluent A) in either polypropylene tubes or a polypropylene 96-well plate. Sufficient Sample Diluent A is provided to perform the following recommended 2-step dilution: (1) Dilute samples 1:10 (1 part sample to 9 parts diluent) by adding 5 µL of sample to 45 µL of 1X Sample Diluent A. (2) Complete the 1:200 sample dilution by performing a 1:20 dilution (1 part of the 1:10 diluted sample to 19 parts diluent) by adding 5 µL of 1:10 diluted sample to 95 µL of 1X Sample Diluent A.

   Note: If you anticipate that your analyte concentrations will be greater than the high point of the calibration curve as reported on the Product Card included in the kit, use prepared 1X Sample Diluent A to further dilute your samples.

3. Incubate prepared calibrator, controls and diluted samples in diluent for 60 minutes prior to adding to the microarrayed plate. This step is crucial for assay accuracy.

4. Following pre-incubation, add 50 µL per well of the calibration curve to duplicate wells of the microarrayed plate.

5. Following pre-incubation, add 50 µL per well of diluted samples and controls to either single or duplicate wells. Load all samples and calibration curve to the plate within 5 minutes.

6. Cover the plate with a provided plate seal, and incubate for 60 minutes at room temperature (20-25°C).

7. Wash the plate 3 times (see Appendix A).

8. Add 50 µL per well of Detection Mix, cover with a new plate seal, and incubate for 30 minutes at room temperature (20-25°C).

9. Wash the plate 3 times (see Appendix A).

10. Add 50 µL per well of Streptavidin-HRP, cover with a new plate seal, and incubate for 20 minutes at room temperature (20-25°C).

11. Wash the plate 6 times (see Appendix A).

12. Add 50 µL per well of previously prepared substrate. Image plate immediately. Wait no longer than 5 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.

13. Place the plate in the Q-View Imager Pro or Q-View Imager LS.
14. Open Q-View Software, create or open a project, and click Acquire Image.
15. When using a Q-View Imager Pro, set the exposure time to 300 seconds.
16. When using a Q-View Imager LS, set the exposure time to 270 seconds and standard image processing.
17. Click the Capture Image(s) button. Users may perform Well Assignment while images are being captured.

    Note: 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**.

18. Dispose of all used and unused materials. Dispose of potentially hazardous waste in accordance with local disposal rules and regulations.

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 Software and Imager Manual, viewable at www.quansysbio.com/manuals, or within Q-View Software under **Support > Manual**.

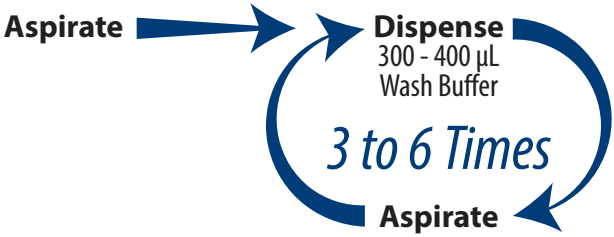
1. Acquire or import an image into Q-View™ Software as previously described.
2. Enter the **Product Code** (found on the Product Card) into the **Product Code** field of the software.
3. **Image Processing**: Align the plate overlay as follows:
    a. Set the overlay: If using the **Auto-Set Plate Overlay** feature, this will occur automatically. Otherwise, go to **Overlay Options > Set Plate Overlay.**
    b. To visualize bright or dim spots, optimize the display using **Image Options > Adjust Gamma** (does not affect the data).
    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.
4. **Well Assignment:** Label wells as samples, controls, calibrators, or negatives and specify their dilution factors. Use **Templates** to quickly assign layouts that are commonly repeated or export the layout as a .csv file.
5. **Data Analysis:** Once **Image Processing** and **Well Assignment** are complete, select **Data Analysis**. Click **Perform Analysis** to generate charts, concentrations, and statistics based on optimal regression settings provided by the product definition.

We take great care to ensure that customers can successfully use 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

Automated Wash 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-450
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 ensure that the wash buffer is running through the plate washer. When the buffer has run through the machine, the waste will be foamy.
6. In a spare microtiter plate, dispense 100 µL wash buffer and ensure that all pins dispense uniformly, then aspirate and ensure that all pins aspirate uniformly. This will ensure that all pins are functioning.
7. Prime the plate washer 1 time before the first wash step. When running the assay, perform the wash 3 or 6 times according to the protocol.

Multichannel Pipette Wash 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 400 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 1 time. When the assay procedure calls for 3 or 6 washes, repeat steps 3-4 accordingly.
6. Tap the plate upside down on a paper towel to remove any residual wash.
7. Proceed immediately to dispense the next solution so drying does not occur.

ABBREVIATED PROTOCOL

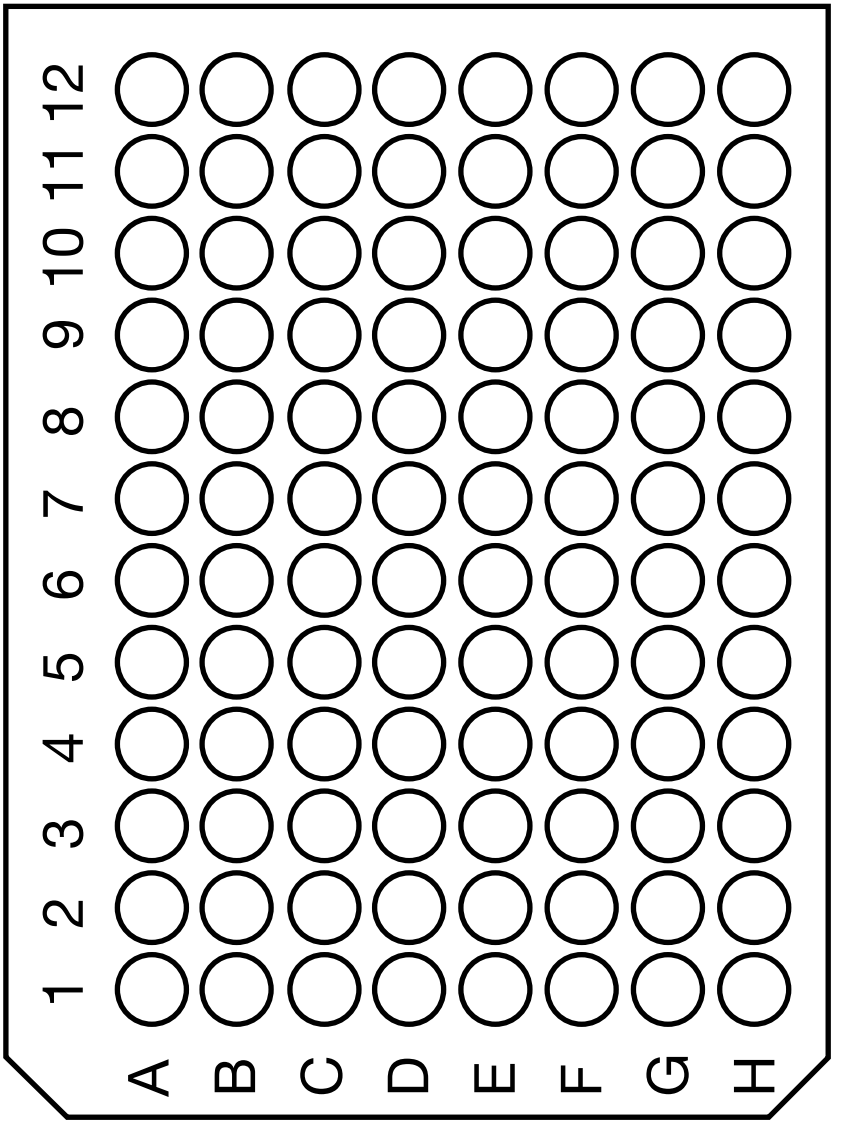
Preparation

1. Install Q-View Software *(page 8)*.
2. Set up the imager *(page 8)*.
3. Set up microplate washer *(page 13).*
4. Reconstitute and prepare reagents *(page 8).*

Running the Assay

5. Prepare the calibration curve using the Calibrator and 1X Sample Diluent A according to the Product Card *(page 9)*.
6. Prepare Controls using 1X Sample Diluent A *(page 8)*.
7. Dilute the samples with 1X Sample Diluent A *(page 10)*.
8. Allow the calibrators, controls and samples to incubate for 60 minutes prior to adding them to the plate *(page 10)*.
9. Load the calibration curve, controls and samples onto the plate. Incubate 60 minutes at room temperature *(page 10)*.
10. Wash the plate 3 times, add the Detection Mix. Incubate 30 minutes at room temperature *(page 10)*.
11. Wash the plate 3 times, add the Streptavidin HRP. Incubate 20 minutes at room temperature *(page 10)*.
12. 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)*.
13. Wash the plate 6 times, and add the mixed Substrate *(page 10)*.
14. Capture and analyze image of the plate *(page 11)*.

PLATE DIAGRAM



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