

Produktinformation



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Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic in





Q-Plex[™]ARRAY Human Malaria 5 Plex Chemiluminescent

For Research Use Only Version 2.8 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		
1	Temperature Limitation		
***	Manufacturer		
茶	Keep Away From Sunlight		

Manufactured and Distributed by:
Quansys Biosciences

365 North 600 West Logan, Utah 84341, 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™ Human Malaria Array (5-plex) Quansys Biosciences Catalog Number 565949HU

The Q-Plex™ Human Malaria Array (5-plex) is a quantitative chemiluminescent assay (ELISA) allowing concurrent measurement in samples of histidine-rich protein 2 (HRP2), lactose dehydrogenase (LDH-Pan, LDH-Pv (Plasmodium vivax), LDH-Pf (Plasmodium falciparum)) and C-reactive protein (CRP) biomarkers in human blood, plasma and serum.

PRINCIPLE OF THE ASSAY

This multiplex assay is based on the microplate sandwich enzyme immunoassay technique for the measurement of HRP2, LDH-Pan, LDH-Pv, and LDH-Pf and the microplate competitive enzyme immunoassay technique for the measurement of CRP.

The HRP2, LDH-Pan, LDH-Pv, and LDH-Pf assays each use two different antibodies specific for their respective targets. Samples or calibrators are pipetted into wells of a microplate arrayed with analyte-specific antibodies that capture HRP2, LDH-Pan, LDH-Pv, and LDH-Pf, thereby immobilizing HRP2, LDH-Pan, LDH-Pv, and LDH-Pf to their locations in the array. After washing away any unbound protein, a mixture that contains biotinylated analyte-specific antibodies is added. The biotinylated antibodies complete the sandwich for each specific arrayed analyte. After washing away unbound biotinylated antibody, streptavidin-horseradish peroxidase (SHRP) is added. Following an additional wash, the amount of SHRP remaining on each location of the array is proportional to the amount of HRP2, LDH-Pan, LDH-Pv, and LDH-Pf initially captured.

The CRP assay uses an antibody specific for its respective target. Samples or calibrators are pipetted into wells of a microplate arrayed with immobilized analyte-specific antibody that captures CRP. During the sample incubation, CRP presents in a sample competing with a fixed amount of biotin-labeled CRP for sites on the immobilized antibody. This is followed by a wash to remove excess biotin-labeled CRP and unbound protein. At this point of the multiplex protocol, the competitive assay acts as a spectator during the biotinylated antibody incubation step of the sandwich assays. After washing

away unbound biotinylated antibody, SHRP is added. Following an additional wash, the amount of SHRP remaining on each location of the array is inversely proportional to the amount of CRP initially present in a sample.

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

Assay Ranges

	Units	Sample Range of Quantification*	Calibrator**
HRP2	pg/ml	675 - 0.6	2,700
LDH-Pan	pg/ml	18,450 - 18	73,800
LDH-Pv	pg/ml	4,925 - 4.8	19,700
LDH-Pf	pg/ml	5,750 - 5.6	23,000
CRP	ng/ml	44,600 - 43.3	44,600

^{*} Based a 1:4 sample dilution

^{**} Actual values may vary from kit to kit. Please see the product card included in your kit for specific values.

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions are intended as general guidelines. Sample stability has not been evaluated.

Whole Blood - Collect blood using EDTA as an anticoagulant. Assay immediately or aliquot and store samples at -20°C to -80°C for single use. Avoid repeated freeze-thaw cycles.

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 to -80°C . Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA 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 to -80°C . Avoid repeated freeze-thaw cycles.

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. 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.
- 5. Do not mix or substitute reagents with those from other kits or lots.
- 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.
- 7. Warning: The calibrator contains 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.

DO

- Do ensure the Q-View[™] Imager Pro or Q-View[™] Imager LS are properly set up following instructions in the imager manual before starting the assay.
- Do set shaker speed to 500 RPM. Shaker performance may vary. Some optimization may be necessary.
- Do dilute all samples, including whole blood, serum and plasma at least 1:4 (one part sample to three parts diluent) with the provided assay diluent to prevent false positives, and mix thoroughly. Higher dilutions may be necessary.
- Pre-wet pipette tips 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 load all calibrators and samples into the microplate within 10 minutes of each other.
- Do be exact with incubation times, particularly the streptavidinhorseradish peroxidase (SHRP) incubation.
- Do be exact when mixing Substrate A and B and mix thoroughly.
- Do image the plate within 5 minutes of adding Substrate using the Q-View™ Imager.

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 - \(\bigset\) 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	2-8°C until kit expiration or at room temperature (20-25°C) for three months	
Assay Diluent Liquid, 6 mL/vial of a buffered protein solution with heterophilic antibody and rheumatoid factor blockers and preservatives	2-8°C until kit expiration	
Calibrator Diluent Lyophilized, of a buffered protein solution containing preservaties.	2-8°C for one week and then discard	
Competitor Lyophilized, biotinylated competitive antigen	Discard unused reconstituted competitor immediately after use	
Detection Mix Liquid, 6 mL/vial of biotinylated antibodies in a buffered protein solution with preservatives	2-8°C until kit expiration	
Calibrator Lyophilized, recombinant antigens in a buffered protein base	Discard unused reconstituted calibrator immediately after use	
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 (3) 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 μ L) and/or a single channel pipette (20-200 μ L) 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. Reservoirs

- Recommended: Biotix disposable reagent reservoirs, SR-0055-BNM
- 4. 5 mL serological pipette
 - a. Recommended: Greiner Bio-One, Pipette, 5 mL, graduated 1/10 ml, 606180
- 5. Micropipettes and pipette tips
- 6. Q-View™ Imager and Software
- 7. Microplate shaker
 - For example: Barnstead/labline 4625 titer plate shaker, IKA MTS 2/4 for 2 or 4 microtiter plates, or equivalent, capable of 500 RPM.
- 8. Deionized water
- 9. Microplate washer
- Graduated cylinder for the preparation of wash buffer and storage container

ASSAY PREPARATION

- Install the 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. Allow all reagents to equilibrate to room temperature (20-25°C) before use. They can remain at room temperature while running the assay.
- 5. Prepare the Wash Buffer: Place 50 mL of the 20X concentrate into 950 mL deionized water, and mix thoroughly.
- 6. Prepare Assay Diluent: Add 1 mL of Assay Diluent to Competitor Mix. Allow Competitor to sit for 5 minutes. Mix thoroughly. Return the 1 mL back into the remaining 5 mL vial of Assay Diluent. Mix thoroughly.
- 7. Prepare Calibrator Diluent: Reconstitute vial using 6 mL deionized water. Allow to sit for 10 minutes. Mix throughly.
- Prepare Calibrator: Reconstitute using Calibrator Diluent with the volume on the Product Card which accompanies the kit. Allow Calibrator to sit for 5 minutes. Mix thorougly.



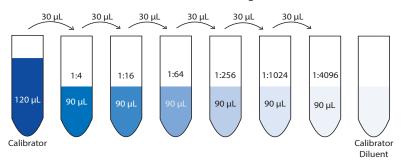
 Allow Substrate A and B to come to room temperature (20-25°C). Fifteen minutes prior to use, combine 3 mL of Substrate A with 3 mL of Substrate B, and mix gently. Do not expose to UV light. Store at room temperature (20-25°C) after mixing.

ASSAY PROCEDURE

It is recommended that all calibrators, samples, and controls be assayed in duplicate.

- 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 120 μL of prepared calibrator into the first tube or well.

- b. Place 90 μL of calibrator diluent into the other seven tubes or wells.
- c. Transfer 30 μ L of the undiluted prepared calibrator from the first tube into the second, mix thoroughly, and repeat the transfer from tube to tube for 6 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 calibrator diluent serves as the negative.



- Prepare Samples: For the recommended sample dilution of 1:4, samples do not need any pre dilution. Samples that require further dilution should be diluted in calibrator diluent prior to diluting in complete assay diluent.
- 3. Prepare Samples and Calibrator: Each point of the calibrator curve and all samples require a 4-fold dilution in the complete assay diluent. Prepare by diluting 1:4 (one part sample to three parts prepared assay diluent) with enough prepared assay diluent to have 50 μL per well in either polypropylene tubes or a polypropylene 96-well plate. For example for two duplicate wells (50 μL each), add 30 μL of calibrator and sample into 90 μL of complete assay diluent.
- 4. Add 50 µL per well of the calibration curve and samples by pre-wetting the pipette tips, then aspirating 50ul and dispensing into the Q-Plex[™] Array 96-well plate. Load all samples and calibration curve to the plate within ten minutes.
- 5. Cover the plate with a plate seal provided, and shake on a plate shaker set to 500 RPM for two hours at room temperature (20-25°C).

- 6. Wash the plate three times (see Appendix A).
- 7. Add 50 μ L per well of Detection Mix, cover with a new plate seal, and return to the plate shaker set to 500 RPM for one hour at room temperature (20-25°C).
- 8. Wash the plate three times (see Appendix A).
- 9. Add 50 μ L per well of Streptavidin-HRP 1X, cover with a new plate seal, and return to the plate shaker set to 500 RPM for 30 minutes at room temperature (20-25°C).
- 10. Wash the plate six times (see Appendix A).

Note: If imaging cannot commence immediately, protect the plate from drying for up to 15 minutes by dispensing 100 μ L 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.

- 11. Add 50μL per well of previously prepared substrate. Wait no longer than 5 minutes to commence imaging.
- 12. Place the plate in the Q-View™ Imager Pro or Q-View™ Imager LS.
- 13. Open Q-View™ Software, create or open a project, and click Acquire Image.
- 14. When using a Q-View™ Imager Pro, set the exposure time to 300 seconds.
- 15. When using a Q-View™ Imager LS, set the exposure time to 270 seconds and standard image processing.
- 16. 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^{TM} Software Manual viewable at www.quansysbio.com/manuals or within Q-View^{TM} Software under **Support > Manual**.

ANALYZING A Q-PLEX™ IMAGE

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

- Acquire or import an image into Q-View[™] Software as described above.
- Enter the **Product Code** (found on the Product Card) into the **Product Code** field.



- 3. Image Processing: Align the plate overlay as follows:
 - To visualize bright or dim spots, optimize the display using Image Options > Adjust Gamma (does not affect the data).
 - 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 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 TECHSUPP@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 μ L) 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 $100\mu L$ 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

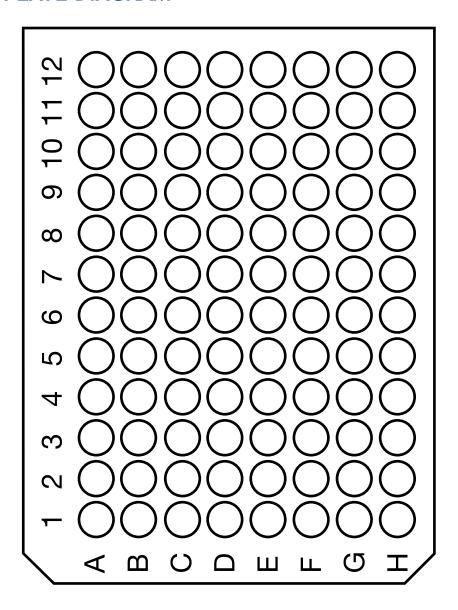
Preparation

- 1. Install Q-View[™] Software (page 8).
- 2. Set up the imager (page 8).
- 3. Set up microplate washer (page 12) and shaker (page 8).
- 4. Reconstitute and prepare reagents (page 8).

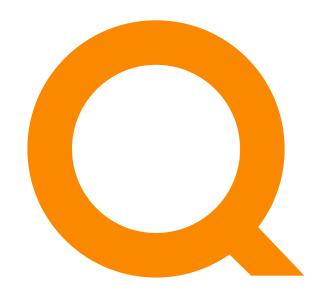
Running the Assay

- 5. Prepare the calibration curve using the Calibrator Diluent according to the Product Card (page 8).
- 6. Prepare the samples and calibrator with complete assay diluent (page 9).
- 7. Load the calibration curve and samples onto the plate. Shake for two hours at room temperature (page 9).
- 8. Wash the plate three times, add the Detection Mix, and shake for one hour at room temperature (page 10).
- 9. Wash the plate three times, add the Streptavidin HRP 1X, shake for 30 minutes at room temperature, (page 10).
- 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 six times, and add the mixed Substrate (page 10).
- 12. Capture and analyze image of the plate (page 10).

PLATE DIAGRAM



NOTES



www.quansysbio.com

365 North 600 West, Logan, Utah 84321

T: 1-888-782-6797 • F: (435)750-6869

www.quansysbio.com • Technical Support: techsupp@quansysbio.com 565917HU