Q-Plex™ Autoantibody Detection 10-Plex Panel

Item No. 502120

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
502121	Q-Plex™ Array Microplate	1 ea	2-8°C
502122	Q-Plex™ Autoantibody Detection Wash Buffer Concentrate (20X)	1 vial/50 ml	2-8°C
502123	Q-Plex™ Autoantibody Detection Sample Diluent (2X)	1 vial/10 ml	2-8°C
502124	Q-Plex™ Autoantibody Detection Mixture	1 ea	2-8°C
502125	Q-Plex™ Autoantibody Detection Negative Control	1 ea	2-8°C
502126	Q-Plex™ Autoantibody Detection Positive Control 1	1 ea	2-8°C
502127	Q-Plex™ Autoantibody Detection Positive Control 2	1 ea	2-8°C
502128	Substrate A	1 vial/3 ml	2-8°C
502129	Substrate B	1 vial/3 ml	2-8°C
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

- Do NOT mix or substitute reagents with those from other kits or lots.
- All products have been 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.
- WARNING: The positive and negative controls contain components of human origin. These components have been tested at the donor level and found negative for hepatitis B surface antigen (HBsAG), HIV-1 and HIV-2 antibodies, and hepatitis C virus (HCV). However, consider all materials as potentially infectious and use only approved guidelines for the proper handling and disposal of infectious material.
- Do NOT allow the plate to dry out between steps.
- DO set up and practice using Q-View™ Imager Pro or Q-View™ Imager LS before starting the assay.
- DO be exact when setting shaker speed to 500 rpm.
- DO dilute all samples 1:100 (1 part sample to 99 parts diluent) with the Sample Diluent (1X) to prevent false positives, and mix thoroughly.
- DO load all samples and controls into the microplate within 10 minutes of each other.
- DO be exact with incubation times, particularly for the Detection Mixture and substrate incubations.
- DO be exact when mixing Substrates A and B. Mix thoroughly. Protect from light.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Q-View™ Imager and software
2. An orbital microplate shaker set at 500 rpm is recommended
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. Deionized water
5. Materials used for **Sample Collection** (see page 12)
6. Microplate washer (optional)

INTRODUCTION

Background

Citrullination and carbamylation are two post-translational modifications (PTMs) that result in the generation of the non-standard amino acids citrulline and homocitrulline, respectively, in polypeptides and proteins.[1] Citrullinated proteins are produced by the deimination of arginine, which is catalyzed by peptidylarginine deiminases (PADs), a family of enzymes with tissue-specific expression patterns. Protein citrullination results in the loss of its positive charge and induces conformational changes that may promote the formation of new protein binding motifs, the generation of neoepitopes, and alter protein function and/or half life.[2] Homocitrulline is produced by the non-enzymatic binding of isocyanic acid to lysine residues resulting in altered structural and functional protein properties.[1,3]

Dysregulation of the citrullination and carbamylation of proteins, including histones, α-enolase, and fibrinogen, has been associated with various inflammatory diseases.[2,4,5] Hypercitrullination of histones leads to the development of neutrophil extracellular traps (NETs), and failure to clear citrullinated proteins and NET components induces the production of autoantibodies, which are associated with a number of human diseases including rheumatoid arthritis, systemic lupus erythematosus, and psoriatic arthritis.[2,6] Unmodified α-enolase is an autoantigen in asthma, Hashimoto's encephalopathy, and rheumatoid arthritis, and autoantibodies against citrullinated α-enolase have been found in the synovial fluid of rheumatoid arthritis patients.[4,7,8] Levels of carbamylated proteins, such as fibrinogen, are increased in several pathologies, including atherosclerosis, chronic kidney disease, and rheumatoid arthritis, and the presence autoantibodies against carbamylated proteins is associated with severe joint damage in patients with rheumatoid arthritis.[5]

Q-Plex™ Multiplex Immunoassay Technology

The Q-Plex™ Autoantibody Detection 10-Plex Panel utilizes Q-Plex™ multiplexing technology from Quansys Biosciences. Q-Plex™ technology accurately detects antibodies and antigens in samples even at low concentrations, providing high specificity and sensitivity using a traditional ELISA protocol. Leveraging an expanded dynamic range, Q-Plex™ decreases the need for multiple dilutions and measures high- and low-abundance analytes in the same assay.

About This Assay

The Q-Plex™ Autoantibody Detection 10-Plex Panel is a qualitative chemiluminescent ELISA allowing concurrent measurement of human antibodies against PAD3, PAD4, fibrinogen, citrullinated fibrinogen, carbamylated fibrinogen, human core histones, citrullinated histones, carbamylated histones, α-enolase, and citrullinated α-enolase in plasma and serum samples.

Principle Of This Assay

This multiplex assay is based on the 96-well plate indirect ELISA technique for the measurement of human antibodies against PAD3, PAD4, fibrinogen, citrullinated fibrinogen, carbamylated fibrinogen, human core histones, citrullinated histones, carbamylated histones, α-enolase, and citrullinated α-enolase.

This assay uses specific proteins to capture the target autoantibodies (see Figure 1, on page 10). Samples or controls are pipetted into wells of a 96-well plate arrayed with PAD3, PAD4, fibrinogen, citrullinated fibrinogen, carbamylated fibrinogen, human core histones, citrullinated histones, carbamylated histones, α-enolase, and citrullinated α-enolase proteins that capture specific autoantibodies, thereby immobilizing them to their locations in the array. After washing away any unbound antibodies, the Detection Mixture that contains an HRP-labeled secondary antibody is added. Following an additional wash, the amount of HRP remaining on each location of the array is proportional to the amount of the initially captured antibodies against PAD3, PAD4, fibrinogen, citrullinated fibrinogen, carbamylated fibrinogen, human core histones, citrullinated histones, carbamylated histones, α-enolase, and citrullinated α-enolase.

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

A schematic of this process is shown in Figure 2, on page 11.

Analyte
PAD3
PAD4
Fibrinogen
Citrullinated Fibrinogen
Carbamylated Fibrinogen
Human Core Histones
Citrullinated Histones
Carbamylated Histones
α-Enolase
Citrullinated α-Enolase
Human IgG Low
Human IgG Medium
Human IgG High
Blank Control

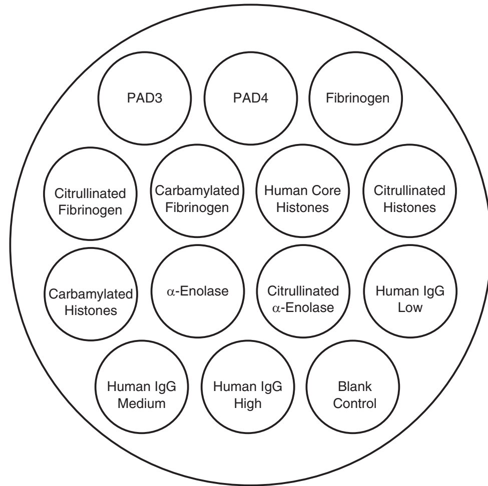

Figure 1. Q-Plex™ Autoantibody Detection 10-Plex Panel well

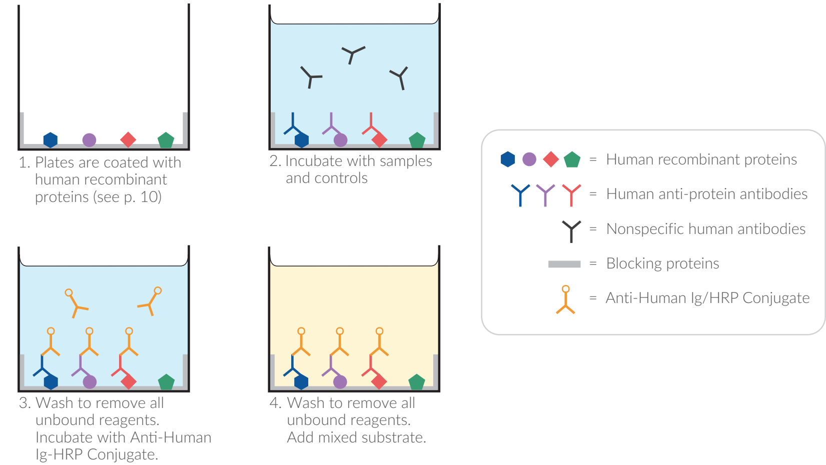

Figure 2. Schematic of the Q-Plex™ Autoantibody Detection 10-Plex Panel assay

PRE-ASSAY PREPARATION

Sample Collection

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

1. **Serum**

   Collect serum in vacutainers or serum separator tubes (SSTs) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1,000-2,000 x g. Pipette off the top yellow serum layer without disturbing the white buffy layer. Assay the serum immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

2. **Plasma**

   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000-2,000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Assay Preparation

Please read the entire section before beginning 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. Visit www.quansysbio.com/manuals for imager specific instructions.
3. Set the orbital plate shaker to 500 rpm.
4. Prepare the Sample Diluent (1X) by mixing equal amounts Sample Diluent (2X) (Item No. 502123) and deionized water.
5. Prepare the Wash Buffer (1X). Add 50 ml of Wash Buffer Concentrate (20X) (Item No. 502122) to 950 ml of deionized water and mix thoroughly.
6. Dilute samples 1:100 by adding 10 µl of sample to 90 µl of Sample Diluent (1X), then adding 20 µl of this solution to 180 µl of Sample Diluent (1X).
7. Reconstitute the Negative Control (Item No. 502125) in 300 µl of Sample Diluent (1X) and Positive Control 1 (Item No. 502126) and Positive Control 2 (Item No. 502127) each in 600 µl of Sample Diluent (1X).
8. The Detection Mixture (Item No. 502124) is ready to use as supplied.
9. Allow Substrates A and B to come to room temperature (20-25°C). Fifteen minutes prior to use, combine 3 ml of Substrate A (Item No. 502128) with 3 ml of Substrate B (Item No. 502129), and mix gently. *NOTE: Do NOT expose to UV light. Store at room temperature (20-25°C) after mixing.*

ASSAY PROTOCOL

Plate Set Up

The 96-well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding the reagents.

Each plate must contain a minimum of two blank (Blk), two non-specific binding (NSB), two negative control (NC), two positive control 1 (PC1), and two positive control 2 (PC2) wells. It is recommended that all samples be assayed in duplicate or triplicate. A suggested plate format is shown in Figure 3, on page 15. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest you record the contents of each well on the template sheet provided (see page 25).'

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	S	S	S	S	S	S	S	S	S	S
B	NSB	NSB	S	S	S	S	S	S	S	S	S	S
C	NC	NC	S	S	S	S	S	S	S	S	S	S
D	PC1	PC1	S	S	S	S	S	S	S	S	S	S
E	PC2	PC2	S	S	S	S	S	S	S	S	S	S
F	S	S	S	S	S	S	S	S	S	S	S	S
G	S	S	S	S	S	S	S	S	S	S	S	S
H	S	S	S	S	S	S	S	S	S	S	S	S

Blk - Blank
NSB - Non-Specific Binding
NC - Negative Control
PC1 - Positive Control 1
PC2 - Positive Control 2
S - Samples

Figure 3. Sample plate format

Performing the Assay

NOTE: 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 samples and controls be assayed in duplicate or triplicate.

1. Equilibrate a pipette tip in a sample or control solution by filling and expelling the liquid back into the original vessel several times. Transfer 50 µl of the sample or control into the appropriate wells on the plate. Use a new tip for each sample/control. Load all samples and controls within 10 minutes. Add 50 µl of Sample Diluent (1X) to the NSB wells. Do not add anything to the Blk wells.
2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate on an orbital microplate shaker at 500 rpm for one hour at room temperature (20-25°C).
3. Wash the plate three times with Wash Buffer (1X) manually or using a plate washer (see Appendix A, on page 21).
4. Add 50 µl of Detection Mixture per well to the sample, control, and NSB wells, cover with a 96-Well Cover Sheet, and return to the plate shaker at 500 rpm for one hour at room temperature (20-25°C).
5. Wash the plate with Wash Buffer (1X) five times manually or using a plate washer (see Appendix A).
6. Add 50 µl per well of the previously prepared mixed substrate to all wells. Begin imaging immediately. *NOTE: If not imaging immediately, do not add mixed substrate. Protect the plate from drying for up to 15 minutes by dispensing 100 µl of Wash Buffer (1X) into each well of the plate. When ready to image, remove the Wash Buffer (1X) from the plate and add the mixed substrate.*
7. Place the plate in the Q-View™ Imager Pro or Q-View™ Imager LS.
8. Open Q-View™ Software, create or open a project, and click Acquire Image.
9. When using the Q-View™ Imager Pro, set the exposure time to 300 seconds. When using the Q-View™ Imager LS, set the exposure time to 270 seconds and standard image processing.
10. Click the Capture Image(s) button.
11. Dispose of all used and unused materials. Disposal of waste may differ from country to country. Please refer to local disposal rules.

Analyzing a Q-Plex™ Image

The following summarizes a general workflow for analyzing a Q-Plex™ image in Q-View™ Software. *Details about these imaging steps are available in the Q-View™ Software Manual available at www.quansysbio.com/manuals or within the 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** 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. Export data (go to **Data Analysis > Export**) and use Excel or another spreadsheet program for analysis.
5. Average all NSB values and calculate the standard deviation. Determine the value for the average NSB plus three standard deviations. This value will be your zero arbitrary ELISA unit (AEU) value and lower limit of detection (LLOD). LLOD is defined as the smallest measure that can be reliably distinguished from the background.
6. When comparing sample values between different plates, it may be useful to normalize each value by the value of the PC1 obtained on the same plate for the same spot. For example, divide the sample value on spot 1 (PAD3) by the value of the PC1 on spot 1 (PAD3) on the same plate, then divide the value of the same sample on spot 2 (PAD4) by the value of the PC1 on spot 2 (PAD4) and so forth. Repeat for each sample on each plate.

Performance Characteristics

Representative Data

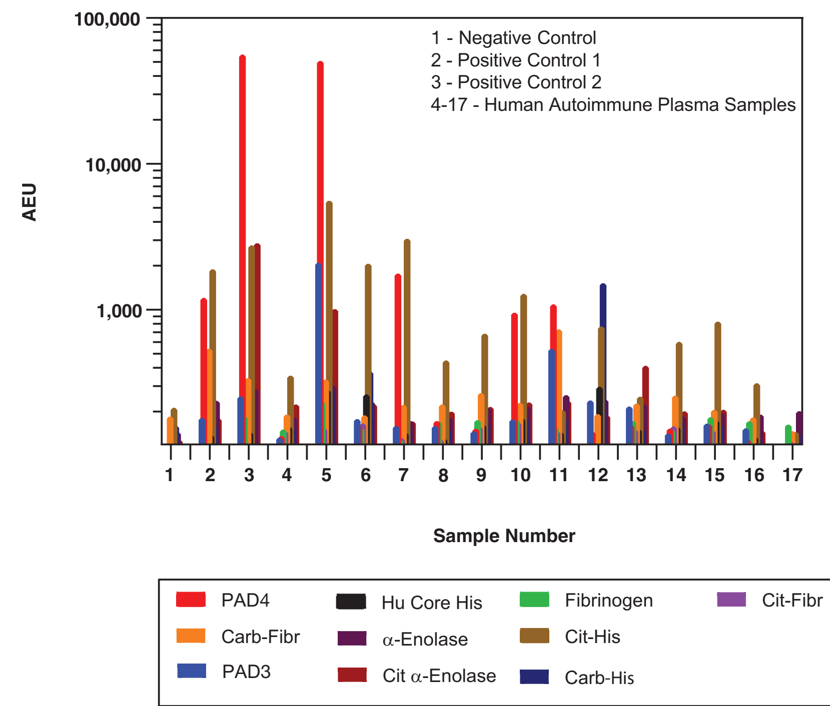

Figure 4. Human autoimmune plasma samples in the Q-Plex™ Autoantibody Detection 10-Plex Panel assay The zero arbitrary ELISA unit (AEU) value corresponds to the average NSB value + three standard deviations. Each sample was tested in three replicates.

Samples	NSB		Negative Control		Positive Control 1		Positive Control 2	
	Value	%CV	Value	%CV	Value	%CV	Value	%CV
PAD3 (Spot 1)	100.7	1.1	104.7	5.4	210.3	16.8	180.7	9.4
PAD4 (Spot 2)	100.0	0.0	101.7	1.5	926.7	14.5	43,254.0	6.8
Fibrinogen (Spot 3)	102.7	4.5	104.3	4.3	102.0	3.4	156.3	17.6
Cit-Fibr (Spot 4)	103.0	5.0	100.0	0.0	107.3	10.3	101.0	1.0
Carb-Fibr (Spot 5)	108.3	7.1	151.7	15.6	504.0	17.3	269.7	24.4
Hu Core His (Spot 6)	100.0	0.0	101.7	2.8	129.0	0.8	116.7	10.4
Cit-Hist (Spot 7)	102.3	2.3	172.7	20.7	2,089.7	4.7	2,378.3	7.3
Carb-Hist (Spot 8)	106.3	8.0	108.3	7.1	156.7	36.8	104.3	6.4
α-Enolase (Spot 9)	106.0	6.8	115.0	3.0	221.3	10.8	254.3	6.9
Cit α-Enolase (Spot 10)	110.7	8.8	124.3	12.2	198.0	16.2	2,718.7	4.6

Table 1. Intra-assay variation in the Q-Plex™ Autoantibody Detection 10-Plex Panel Each control was tested in triplicate.

Appendix A: Plate Washing Method

1. Use a program that will aspirate and dispense 300-400 µl Wash Buffer (1X).

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 protein arrays. The simplest method to avoid plate drying is to leave a small, uniform volume (1-3 µl) of Wash Buffer (1X) in the well after the final aspiration and add the next reagent to the plate as quickly as possible.
3. Leaving a uniform volume of the Wash Buffer (1X) in the wells can be accomplished with an automated washer by slightly increasing the aspiration height of the washer head. See examples below:

Process	Distance	Steps on a Biotek ELX-405
Aspiration Height	3.81 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 (1X) to the automatic plate washer.
5. Run 1-2 priming cycles to make sure that the Wash Buffer (1X) 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 microplate dispense 100 µl Wash Buffer (1X) 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 three to six times according to the protocol.

ABBREVIATED PROTOCOL

Preparation

1. Install Q-View™ Software (see page 13).
2. Set up the imager (see page 13).
3. Set up microplate washer (optional, see page 21) and shaker (see page 13).
4. Prepare reagents (see page 13).

Running the Assay

5. Dilute the samples with Sample Diluent (1X) (see page 16).
6. Load samples and controls onto the plate. Load Sample Diluent (1X) into NSB wells. Shake for one hour at room temperature (500 rpm) (see page 16).
7. Wash the plate three times, add the Detection Mixture to all wells except Blk wells, and shake for one hour at room temperature (500 rpm) (see page 16).
8. Allow Substrates A and B to come to room temperature, then mix equal volumes and allow the solution to incubate at room temperature for 15 minutes prior to addition to the plate. (see pages 13-18).
9. Wash the plate five times and add the mixed substrate to wells (see page 16 and 21).
10. Capture and analyze image(s) of the plate (see pages 16-18).

RESOURCES

References

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

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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