Quantibody® Rat Cytokine Antibody Array 282

A combination of 8 non-overlapping arrays to quantitatively measure 282 rat cytokines

Catalog #: QAR-CAA-282

User Manual
Last revised December 17, 2021

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

ISO 13485 Certified

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (282)	Arrays Included: QAR-CYT-3 (27); QAR-CYT-4 (40); QAR-CYT-5 (40); QAR-CYT-6 (40); QAR-CYT-7 (40); QAR-CYT-8 (40); QAR-CYT-9 (40); QAR-SAP-1 (15) *See Section IX for Array Map*
Format	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
Detection Method	Fluorescence. Go to www.RayBiotech.com/Scanners for a list of compatible laser scanners.
Sample Volume	50 - 100 µl per array
Reproducibility	CV <20%
Assay Duration	6 hours

II. Introduction

Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in interactions between different cell types, cellular responses to environmental conditions, and maintenance of homeostasis. In addition, cytokines are also involved in most disease processes, including cancer and cardiac diseases.

The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent assay (ELISA). In this method, target protein is immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the enzyme complex can then be visualized through the use of a substrate that produces a detectable signal. While this traditional method works well for a single protein, the overall procedure is time consuming and requires a relatively high volume of sample. Thus, conservation of precious small sample quantities becomes a challenging task. Innovations in microarray technology over the last decade have addressed this problem. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which have now been widely applied in the research community with hundreds of peer reviewed publications, including top-tier journals such as *Cell* and *Nature*.

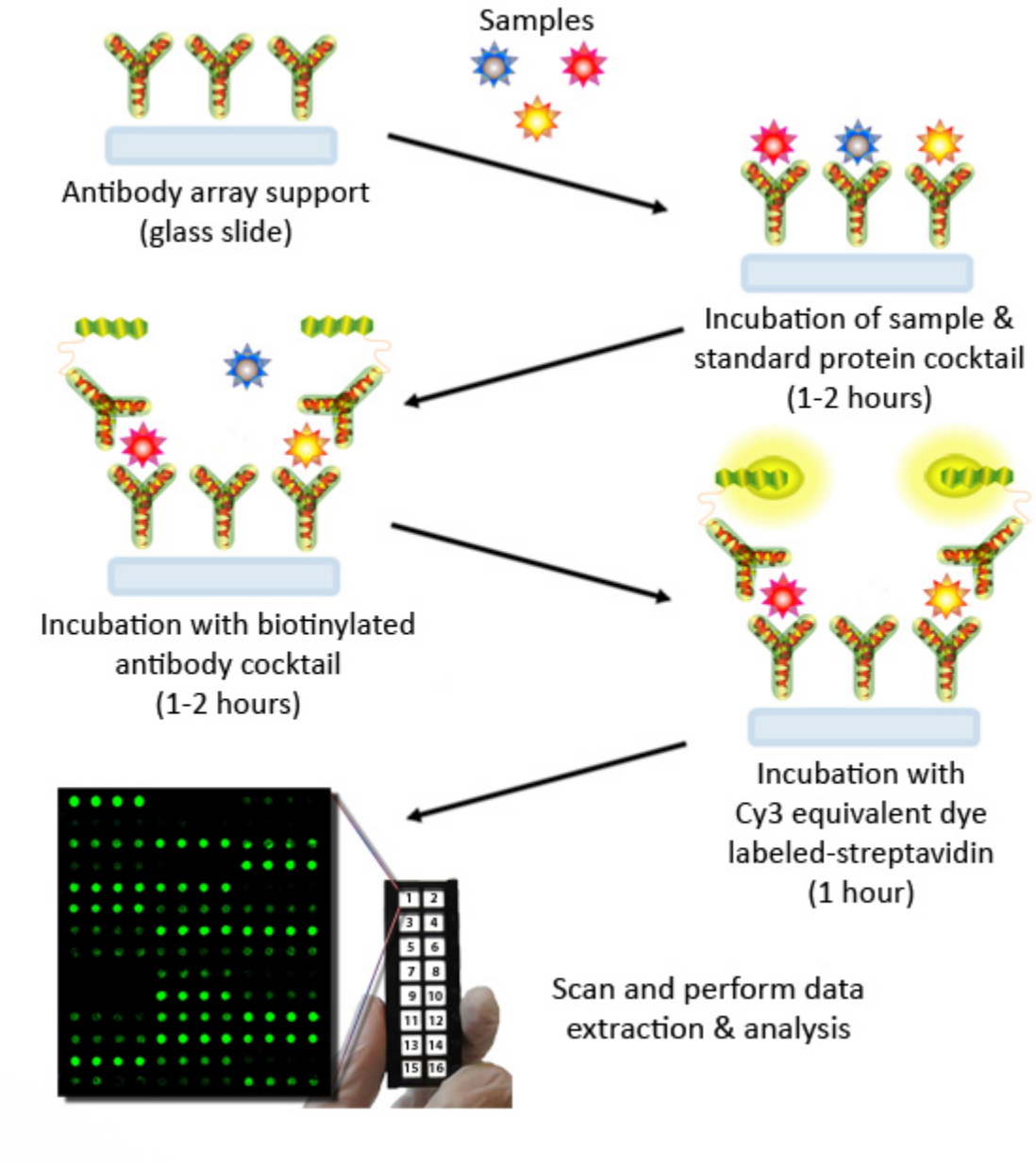
The Quantibody® array, our multiplexed sandwich ELISA-based quantitative array platform, enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and the high throughput of arrays. Like a traditional sandwich-based ELISA, it uses a pair of cytokine specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target cytokine is

trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different epitope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-conjugated Cy3 equivalent dye, using a laser scanner. Unlike the traditional ELISA, Quantibody products use an array format. By arraying multiple cytokine specific capture antibodies onto a glass support, quantitative, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is divided into 16 wells of identical cytokine antibody arrays. Each antibody, together with the positive controls is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples on one slide. Four slides can be nested into a tray, which matches a standard microplate footprint and allows for automated robotic high throughput process of 64 arrays simultaneously. For cytokine quantification, the array specific cytokine standards, whose concentration has been predetermined, are provided to generate a standard curve for each cytokine. In a real experiment, standard cytokines and samples will be assayed in each array simultaneously through a sandwich ELISA procedure. By comparing signals from unknown samples to the standard curve, the cytokine concentration in the samples will be determined.

Quantibody® array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Our current high density Quantibody kits allow scientists to quantitatively determine the concentration of 1000 human, 200 mouse, and 67 rat cytokines in a single experiment. This is not only one of the most efficient products on the market for cytokine quantification, but makes it more affordable for quantification of large number of proteins. Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for drug and biomarker discovery.

III. How It Works



IV. Materials Provided

This product is a combination of multiple arrays. Items 1, 5, & 6 are array-specific.

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	*[Array-Cat-#]* S	*Array-specific* Glass Slide	1	2
2	QA-SDB	Quantibody® Sample Diluent	15 ml	
3	AA-WB1-30ML	20X Wash Buffer I	2 x 30 ml	3 x 30 ml
4	AA-WB2-30ML	20X Wash Buffer II	30 ml	
5	*[Array-Cat-#]* -STD	*Array-specific* Lyophilized Standard Mix**	1 Vial	
6	*[Array-Cat-#]* B	*Array-specific* Biotinylated Antibody Cocktail	1-25 µl	2 x 1-25 µl
7	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 µl	2 x 5 µl
8	QA-SWD	Slide Washer/Dryer	1 x 30 ml Tube	
9	QA-ADH	Adhesive Film	1	2

* 4 slide kits are comprised of 2 separate 2 slide kits.
** See Section X for detailed cytokine concentrations after reconstitution.

V. Storage

Upon receipt, all components should be stored at -20°C. The kit will retain activity for up to 6 months. Once thawed, the glass slide, standard mix, antibody cocktail and dye-conjugated Streptavidin should be kept at -20°C. All other components may be stored at 4°C. The entire kit should be used within 6 months of purchase.

VI. Additional Materials Required

- Benchtop rocker or orbital rocker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- 1.5 ml Polypropylene microcentrifuge tubes

VII. General Considerations

A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contains cytokines.
- Each array needs 100 µl of total sample volume. To avoid matrix effects, we recommend using a minimum of 2-fold sample dilution of culture media, body fluids, or 0.5-1mg/ml total protein for lysates, after a 5-fold to 10-fold dilution to minimize the effects of any detergent(s). Please be aware, more sample volume is required for combination arrays. For example, the minimum sample volume for a 10-array kit is 500 µl, or 500 µg lysate.
- The suggested serum/plasma dilution for this array is: 2x; 1000x (QAR-SAP-1)

B. Handling Glass Slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with powder free gloves.
- Handle glass slide/s in clean environment.
- Permanent marker ink can significantly interfere with fluorescent signal detection. To help distinguish one slide from another, you may make a small marking (such as a number or a star) along the top or bottom edge, using a green or blue ultra-fine point Sharpie® brand marker. This can also serve to orient the slide. For best results during scanning, please **DO NOT**:
    - Write anywhere on the front (arrayed) side of the slide
    - Write on the slide while it is wet
    - Use red or black colored ink anywhere on the slide
    - Write over the arrayed well areas of the slide, as this interferes with scanning.

C. Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rocking or rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µl of sample or reagent is used.

- Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

VIII. Protocol

Note: *This product contains sets of reagents for different arrays. Always ensure you are using the proper glass slide, lyophilized standard mix, and biotinylated antibody cocktail for the correct corresponding array.*
The following procedure is for processing any one of the arrays in the kit.

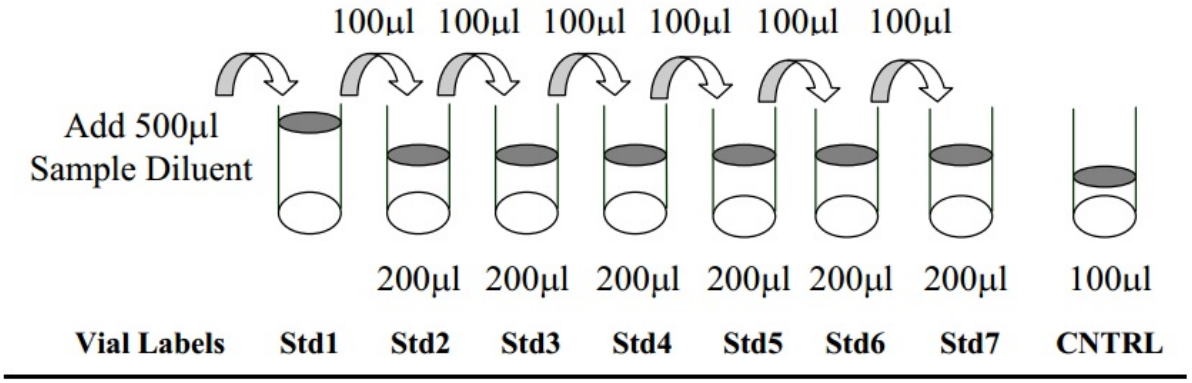
A. Completely Air Dry The Glass Slide

1. Take out the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag, peel off the cover film, and let it air dry for another 1-2 hours.

Incomplete drying of slides before use may cause the formation of "comet tails," thin directional smearing of antibody spots.

B. Prepare Cytokine Standard Dilutions

There is only one vial of standard provided in the two-slide kit, which is enough for making two standard curves. Reconstitute the lyophilized standard within one hour of usage. If you must use the standard for two different days, store only the Std1 dilution at -80°C.



2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500 µl Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1.

3. Label 6 clean microcentrifuge tubes as Std2 to Std7. Add 200 µl Sample Diluent to each of the tubes.
4. Pipette 100 µl Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100 µl Std2 to tube Std3 and so on.
5. Add 100 µl Sample Diluent to another tube labeled as CNTRL. Do not add standard cytokines or samples to the CNTRL tube, which will be used as negative control. For best results, include a set of standards in each slide.

Since the starting concentration of each cytokine is different, the serial concentrations from Std1 to Std7 for each cytokine are varied which can be found in Section X.

C. Blocking & Incubation

6. Add 100 µl Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.
7. Decant buffer from each well. Add 100 µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour.

Longer incubation time is preferable for higher signals. This step may be done overnight at 4°C.

We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 250 µg/ml-1 mg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation, especially if less than 70 ul of sample or reagent is used.

8. Wash:

- Decant the samples from each well, and wash 5 times (5 min each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with H2O.

- *(Optional for Cell and Tissue Lysates)* Put the glass slide with frame into a box with 1X Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle rocking for 20 min.

- Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 150 µl of 1X Wash Buffer II at room temperature with gentle rocking.

Completely remove wash buffer in each wash step. Dilute 20X Wash Buffer II with H2O.

Incomplete removal of the wash buffer in each wash step may cause "dark spots," the background signals higher than the spots.

D. Incubation with Biotinylated Antibody Cocktail & Wash

9. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.
10. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour.

Longer incubation time is preferable for higher signals and backgrounds

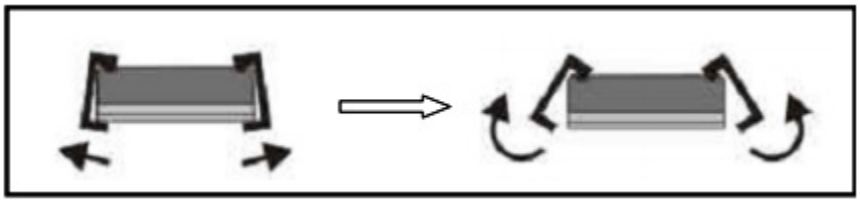
11. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I and then 2 times with 150 µl of 1x Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

E. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
13. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.
14. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

F. Fluorescence Detection

15. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.



Be careful not to touch the surface of the array side.

16. Place the slide in the Slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) and gently shake at room temperature for 5 minutes.
17. Remove water droplets completely by gently applying suction with a pipette to remove water droplets. Do not touch the array, only the sides.

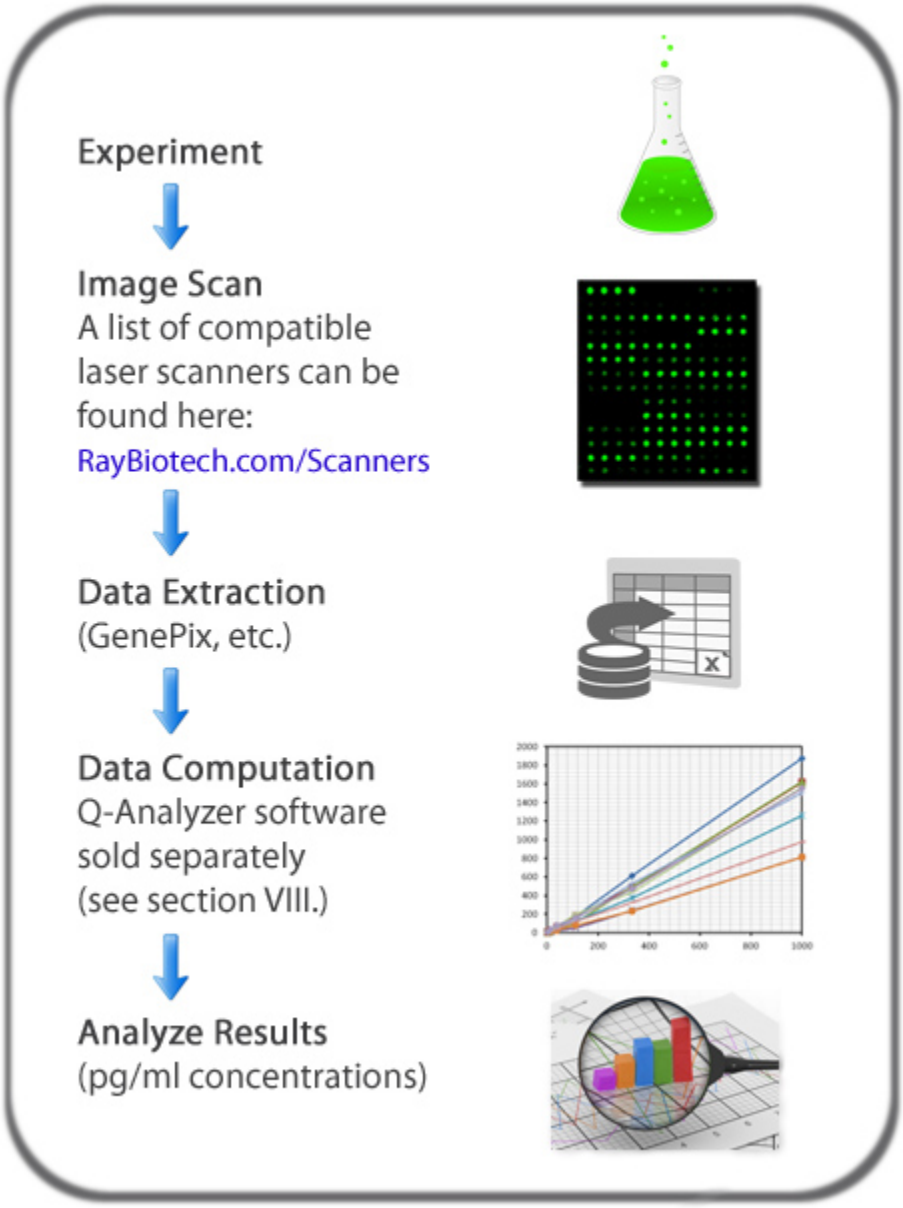
    You may also dry the glass slide by a compressed N_2 stream.
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains unsaturated.

In case the signal intensity for different cytokine varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal cytokines, and a low PMT for high signal cytokines.

G. Data Analysis

19. Data extraction can be done using the GAL file that is specific for this array (QAR-CAA-282) along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). The GAL file can be found on the product web page under the 'Files' tab.

    Need help analyzing all that data? All RayBiotech array analysis tools are now free to download! Just like the GAL file, you can find this 'Q-Analyzer' tool on the product web page under the 'Files' tab. More information can be found in Section XII.



IX. Array Map & Standard Curves

Please view the individual array manuals for representative standard curve images

QAR-CYT-3

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				NEG			
B	CD86 (B7-2)				beta-NGF				CINC-1 (CXCL1)			
C	CINC-2				CINC-3				CNTF			
D	Fractalkine				GM-CSF				ICAM-1 (CD54)			
E	IFN-gamma				IL-1 alpha				IL-1 beta			
F	IL-2				IL-4				IL-6			
G	IL-10				IL-13				LIX			
H	L-Selectin				MCP-1				PDGF-AA			
I	Prolactin R				RAGE				TCK-1 (CXCL7)			
J	TIMP-1				TNF-alpha				VEGF-A			

QAR-CYT-4

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				4-1BB (CD137)			
B	Activin A				Adiponectin				CD80 (B7-1)			
C	CD48 (SLAMF2)				CTACK (CCL27)				Decorin			
D	Eotaxin-1				EphA5				Erythropoietin			
E	FGF-BP				Flt-3 Ligand				Galectin-1			
F	Galectin-3				Gas 1				GFR alpha-1			
G	gp130				HGF				IL-1 R6			
H	IL-1 ra (IL-1 F3)				IL-2 R alpha				IL-3			
I	IL-7				IL-17F				IL-22			
J	JAM-A				MIP-1 alpha				Neuropilin-1			
K	Neuropilin-2				Nope				Notch-1			
L	Notch-2				P-Cadherin				Prolactin			
M	RANTES (CCL5)				SCF				TIM-1 (KIM-1)			
N	TIMP-2				TREM-1				TWEAK R			

QAR-CYT-5

(rCYT-5 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				4-1BB Ligand			
B	ACE2				Activin R2A				ADAM9			
C	AGT				ANG-2				APLP-1			
D	ASAH2				BAFF				BDNF			
E	BID				BMP-7				C4.4A			
F	CA14				Cadherin-4				CADM3			
G	Cathepsin E				Cathepsin L				CD14			
H	CD226				CD28				CD34			
I	CD36				CD38				CD40			
J	CD44				CD47				CD59			
K	CD5L				CD93				CDH5			
L	CDNF				CEACAM1				CLEC5A			
M	CNTN1				CNTN3				Contactin-4			
N	CPA1				CPB1				CRELD1			

QAR-CYT-6

(rCYT-6 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				CSF1R			
B	CXCL16				Cystatin C				Dkk-3			
C	Endoglin				EpCAM				EphB6			
D	Ephrin-A2				E-Selectin				F3			
E	FABP4				Fas				Fas Ligand			
F	FCAR				FCGR2B				FCGRT			
G	FETUB				FGF-12				FGFR4			
H	Follistatin				GDF-3				GDNF			
I	GFR alpha-2				GFR alpha-3				GGT1			
J	HS6ST3				IDS				IFNA5			
K	IFN-gamma R2				IGSF8				IL-11 RA			
L	IL-17 RC				IL-1 R2				IL-20 RB			
M	IL-21				IL-21 R				IL-2 RG			
N	IL-31				IL-5				IL-7 R			

QAR-CYT-7

(rCYT-7 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				IL-9			
B	IL-9 R				Jagged 1				JAM-C			
C	KDR				KLKB1				Klotho beta			
D	Layilin				Legumain				Leptin			
E	LIF				LRPAP				LTBR			
F	LTF				Lumican				MAG			
G	Matrilin-4				MCP-3				MSP			
H	Nephrin				Neurexin 1 alpha				Neurexophilin-3			
I	Neuroplastin				Noggin				Notch-3			
J	Osteopontin				OX40				PDGF R alpha			
K	PDGF-BB				PGC				pIgR			
L	Plexin A1				PLTP				Pref-1			
M	PVRL1				RBP4				Reg3A			
N	Renin 1				RGM-B				ROBO1			

QAR-CYT-8

(rCYT-8 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAMTS1			
B	DLL1				GDF-8				IL-12			
C	IL-18				MESDC2				MIP-1b			
D	MMP-2				Neurexophilin-1				Neuroligin 1			
E	NPC2				NT5E				Olfactomedin-1			
F	PROCR				Protocadherin-12				P-selectin			
G	PVR				Reg3B				REG4			
H	SCGF				SEMA3F				SEMA4D			
I	SEMA7A				Serpin A3N				Serpin D1			
J	Serpin F2				SGSH				SIGNR1			
K	SIRP alpha				STX7				Syndecan-1			
L	Syndecan-3				TGF-beta RIII				TNFRSF11A			
M	TrkB				Trypsin 3				UCHL1			
N	UNC5H1				VEGFR1				XPNPEP2			

QAR-CYT-9

(rCYT-9 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				aFGF			
B	ALCAM				Ameloblastin				BAMBI			
C	BST1				Cathepsin B				Cathepsin C			
D	CD164				CD1d1				CD300LG			
E	CD5				CD6				CD83			
F	CLEC4B2				COPZ1				CPA2			
G	CRELD2				DSC2				ECM-1			
H	EGF				EGF R				EphA3			
I	EphB3				ERBB3				FCGR3A			
J	FGF-21				FOLR1				FSTL1			
K	GITR				IGFBP-1				IL-13 Ra2			
L	IL-15 R alpha				IL-17RA				IL-1 R1			
M	IL-4 R				Serpin E1				Serpin F1			
N	Sortilin				SP-D				TrkA			

QAR-SAP-1

QAR-SAP-1 Map: Each antibody is printed in quadruplicate								
	1	2	3	4	1	2	3	4
A	POS1				POS2			
B	APOH				B2M			
C	Clusterin				CRP			
D	HPX				IGF-1			
E	KNG1				Lipocalin-2			
F	MBL-2				Pentraxin 2			
G	PGRP-S				Protein C			
H	PSAP				Serpin A1c			
I	Transferrin				Blank			

X. Standard Concentrations

After reconstitution, the lyophilized cytokine standard mix contains the following concentrations for each antigen included.

QAR-CYT-3	(pg/ml)	QAR-CYT-4	(pg/ml)	QAR-CYT-5	(pg/ml)	QAR-CYT-6	(pg/ml)
B7-2	10,000	4-1BB	200,000	4-1BB Ligand	20,000	CSF1R	100,000
b-NGF	10,000	Activin A	40,000	ACE2	100,000	CXCL16	20,000
CINC-1	20,000	Adiponectin	10,000	Activin R2A	20,000	Cystatin C	4,000
CINC-2	10,000	B7-1	40,000	ADAM9	4,000	Dkk-3	2,000
CINC-3	10,000	CD48	2,000	AGT	20,000	Endoglin	20,000
CNTF	20,000	CTACK	200,000	ANG-2	40,000	EpCAM	10,000
Fractalkine	20,000	Decorin	20,000	APLP-1	1,000	EphB6	2,000
GM-CSF	10,000	Eotaxin-1	1,000	ASAH2	4,000	Ephrin-A2	2,000
ICAM-1	20,000	EphA5	10,000	BAFF	1,000	E-Selectin	200
IFNg	4,000	Erythropoietin	200,000	BDNF	1,000	F3	100,000
IL-10	50,000	FGF-BP	40,000	BID	10,000	FABP4	10,000
IL-13	10,000	Flt-3 Ligand	100,000	BMP-7	4,000	Fas	10,000
IL-1a	10,000	Galectin-1	20,000	C4.4A	2,000	Fas Ligand	100,000
IL-1b	10,000	Galectin-3	4,000	CA14	2,000	FCAR	4,000
IL-2	10,000	Gas 1	4,000	Cadherin-4	10,000	FCGR2B	10,000
IL-4	200	GFR alpha-1	100,000	CADM3	1,000	FCGRT	10,000
IL-6	10,000	gp130	100,000	Cathepsin E	4,000	FETUB	1,000
LIX	40,000	HGF	20,000	Cathepsin L	10,000	FGF-12	10,000
L-Selectin	10,000	IL-1 R6	100,000	CD14	4,000	FGFR4	4,000
MCP-1	2,000	IL-1ra	40,000	CD226	100,000	Follistatin	2,000
PDGF-AA	20,000	IL-17F	2,000	CD28	40,000	GDF-3	40,000
Prolactin R	40,000	IL-2 Ra	40,000	CD34	10,000	GDNF	4,000
RAGE	20,000	IL-22	40,000	CD36	100,000	GFR alpha-2	4,000
TCK-1	10,000	IL-3	40,000	CD38	4,000	GFR alpha-3	4,000
TIMP-1	10,000	IL-7	40,000	CD40	40,000	GGT1	10,000
TNFa	100,000	JAM-A	20,000	CD44	10,000	HS6ST3	10,000
VEGF-A	1,000	MIP-1a	40,000	CD47	10,000	IDS	100,000
		Neuropilin-1	40,000	CD59	200	IFNA5	4,000
		Neuropilin-2	100,000	CD5L	2,000	IFN-gamma R2	1,000
		Nope	10,000	CD93	40,000	IGSF8	4,000
		Notch-1	4,000	CDH5	4,000	IL-11 R alpha	4,000
		Notch-2	4,000	CDNF	1,000	IL-17 RC	4,000
		P-Cadherin	4,000	CEACAM1	40,000	IL-1 R2	4,000
		Prolactin	20,000	CLEC5A	10,000	IL-20 RB	10,000
		RANTES	4,000	Contactin-1	10,000	IL-21	20,000
		SCF	4,000	Contactin-3	100,000	IL-21 R	20,000
		TIM-1	40,000	Contactin-4	10,000	IL-2 RG	10,000
		TIMP-2	10,000	CPA1	100,000	IL-31	4,000
		TREM-1	20,000	CPB1	10,000	IL-5	10,000
		TWEAK R	100,000	CRELD1	4,000	IL-7 R	4,000

QAR-CYT-7	(pg/ml)	QAR-CYT-8	(pg/ml)	QAR-CYT-9	(pg/ml)	QAR-SAP-1	(pg/ml)
IL-9	100,000	ADAMTS1	20,000	aFGF	20,000	APOH	10,000
IL-9 R	20,000	DLL1	100,000	ALCAM	10,000	B2M	2,000
Jagged 1	4,000	GDF-8	100,000	Ameloblastin	10,000	Clusterin	20,000
JAM-C	2,000	IL-12	40,000	BAMBI	10,000	CRP	20,000
KDR	20,000	IL-18	100,000	BST1	100,000	HPX	20,000
KLKB1	10,000	MESDC2	2,000	Cathepsin B	2,000	IGF-1	10,000
Klotho beta	10,000	MIP-1b	1,000	Cathepsin C	40,000	KNG1	10,000
Layilin	1,000	MMP-2	20,000	CD164	40,000	Lipocalin-2	20,000
Legumain	4,000	Neurexophilin-1	10,000	CD1d1	100,000	MBL-2	4,000
Leptin	20,000	Neuroligin 1	100,000	CD300LG	100,000	Pentraxin 2	10,000
LIF	1,000	NPC2	10,000	CD5	10,000	PGRP-S	4,000
LRPAP	10,000	NT5E	20,000	CD6	40,000	Protein C	10,000
LTBR	20,000	Olfactomedin-1	20,000	CD83	20,000	PSAP	2,000
LTF	10,000	PROCR	10,000	CLEC4B2	40,000	Serpin A1c	10,000
Lumican	4,000	Protocadherin-12	100,000	COPZ1	100,000	Transferrin	20,000
MAG	1,000	P-selectin	20,000	CPA2	40,000		
Matrilin-4	1,000	PVR	100,000	CRELD2	4,000		
MCP-3	400	Reg3B	1,000	DSC2	10,000		
MSP	2,000	REG4	1,000	ECM-1	10,000		
Nephrin	10,000	SCGF	20,000	EGF	1,000		
Neurexin 1 alpha	100,000	SEMA3F	10,000	EGF R	4,000		
Neurexophilin-3	2,000	SEMA4D	20,000	EphA3	4,000		
Neuroplastin	10,000	SEMA7A	10,000	EphB3	100,000		
Noggin	20,000	Serpin A3N	20,000	ERBB3	100,000		
Notch-3	4,000	Serpin D1	20,000	FCGR3A	10,000		
Osteopontin	2,000	Serpin F2	40,000	FGF-21	1,000		
OX40	2,000	SGSH	10,000	FOLR1	10,000		
PDGF R alpha	10,000	SIGNR1	10,000	FSTL1	10,000		
PDGF-BB	4,000	SIRP alpha	10,000	GITR	4,000		
PGC	20,000	STX7	1,000	IGFBP-1	1,000		
pIgR	2,000	Syndecan-1	10,000	IL-13 Ra2	400		
Plexin A1	20,000	Syndecan-3	2,000	IL-15 R alpha	4,000		
PLTP	10,000	TGF-beta RIII	10,000	IL-17RA	20,000		
Pref-1	1,000	TNFRSF11A	1,000	IL-1 R1	40,000		
PVRL1	100,000	TrkB	2,000	IL-4 R	1,000		
RBP4	100,000	Trypsin 3	10,000	Serpin E1	40,000		
Reg3A	1,000	UCHL1	10,000	Serpin F1	20,000		
Renin 1	1,000	UNC5H1	10,000	Sortilin	20,000		
RGM-B	2,000	VEGFR1	10,000	SP-D	100,000		
ROBO1	40,000	XPNPEP2	10,000	TrkA	10,000		

XI. Spiking & Recovery

Please view the individual array manuals for spiking & recovery data

XII. Quantibody® Q-Analyzer

The Q-Analyzer tool can be downloaded from the 'Files' tab on the product web page for this array: **QAR-CAA-282**.

The Q-Analyzer is an array specific, Excel-based program. It is much more than a simple calculation macro; it performs sophisticated data analysis (see below for description).

Key features:

- Simplicity: Easy to operate and requires no professional training. With a simple copy and paste process, the cytokine concentration is determined.
- Outlier Marking & Removing: The software can automatically mark and remove the outlier spots for more accurate data analysis
- Normalization: The program allows for intra- and inter-slide normalization for large numbers of samples.
- Two Positive Controls: The program utilizes the two positive controls in each array for normalization.
- Two Analytical Algorithms: Users can choose either linear regression or log-log algorithms to meet their analytical needs.
- Two Data Outputs: standard curves and digital concentration.
- User Intervention: The program allows for user manual handling of outliers and other analytical data.
- Lower and Upper Limits Determination: The program automatically marks out the values below or above the detection range.
- Standard Deviation: The program outputs the standard deviations of the quadruplicate spots for data accuracy.
- Analytical Tips: Q-Analyzer analysis tips are included in the program.

XIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Increase incubation time or change sample incubation step to overnight
	Too low protein concentration in sample	Lessen dilution or do not dilute sample. Concentrate sample if necessary.
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Decrease amount of rocking during incubations. check for bubble formation and remove bubbles.
	Arrays are not completed covered by reagent	Completely cover arrays with solution for all required steps.
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer and other solutions into neighboring wells.
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard well at the room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions.
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated.
	Use freeze-thawed cytokine standards	Always use new cytokine standard vial for new set of experiment. Discard any leftover.
High background	Overexposure	Lower the PMT or signal gain.
	Dark spots	Completely remove wash buffer in each wash step.
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Work in clean environment
	Slide is allowed to dry out	Don't dry out slides during experiment.

XIV. Select Quantibody® Publications

1. Zeng Q., et al. The functional behavior of a macrophage/fibroblast co-culture model derived from normal and diabetic mice with a marine gelatin-oxidized alginate hydrogel. Biomaterials. 2010 Aug;31(22):5772-81. doi: 10.1016/j.biomaterials.2010.04.022.
   Species: Mouse
2. Toh H, Wang W, Chia W, Kvistborg P, Sun Li,et al. Clinical Benefit of Allogeneic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients.Clin Cancer Res. 2009;15(24):7726-7736
   Species: Human
   Sample Type: Plasma
3. Du Y, Wei X, He Y, Wei G, Hampel H, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. Alzheimer Dementia. 2008;4(4 Suppl):T484 (Abstract P2-380).
   Species: Human
   Sample Type: Plasma
4. Jonnalagadda D., et al. Platelet secretion is kinetically heterogeneous in an agonist-responsive manner. December 20, 2012; Blood: 120 (26). http://dx.doi.org/10.1182/blood-2012-07-445080
   Species: Human
   Sample Type: Conditioned Media
5. Vargas-Inchaustegui D., Hogg A., Tulliano G., et al.CXCL10 Production by Human Monocytes in Response to Leishmania braziliensis Infection. Infect. Immun. January 2010 vol. 78 no. 1 301-308
   Species: Human
   Sample Type: Serum
6. Zhai Y, Zhong Z, Chen C-YA, Xia Z, Song L, Blackburn MR, Shyu A-B. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. Mol Cell Biol. 2008; 28(24):7414-7426.
   Species: Human
7. Huggenberger R., et al. Stimulation of lymphangiogenesis via VEGFR-3 inhibits chronic skin inflammation. J Exp Med. 2010 Sep 27;207(10):2255-69. doi: 10.1084/jem.20100559.
   Species: Mouse
   Sample Type: Tissue Lysate
8. Jurk D., Wilson C., Passos J., et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nature Communications 2, Article number: 4172. doi:10.1038/ncomms5172
   Species: Mouse
   Sample Type: Conditioned Media
9. Bethunaickan, R., Sahu, R., Liu, Z., Tang, Y. T., Huang, W., Edegbe, O., Tao, H., Ramanujam, M., Madaio, M. P. and Davidson, A. (2012), Anti-tumor necrosis factor alpha treatment of interferon-alpha-induced murine lupus nephritis reduces the renal macrophage response but does not alter glomerular immune complex formation. Arthritis & Rheumatism, 64: 3399-3408. doi: 10.1002/art.34553
   Species: Mouse
   Sample Type: Tissue Lysate
10. Hou T., Li Z., Luo F., Xie Z., Wu X., Xing J., Dong S., Xu J. A composite demineralized bone matrix e Self assembling peptide scaffold for enhancing cell and growth factor activity in bone marrow. Biomaterials, Available online 19 April 2014. [Epub ahead of print]
    Species: Mouse
    Sample Type: Tissue Lysate
11. Feng W., Madajka M., Kerr B., Mahabeleshwar G., White S., Byzova T. A novel role for platelet secretion in angiogenesis: mediating bone marrow-derived cell mobilization and homing. Blood April 7, 2011 vol. 117 no. 14 3893-3902
    Species: Mouse

XV. Experiment Record Form

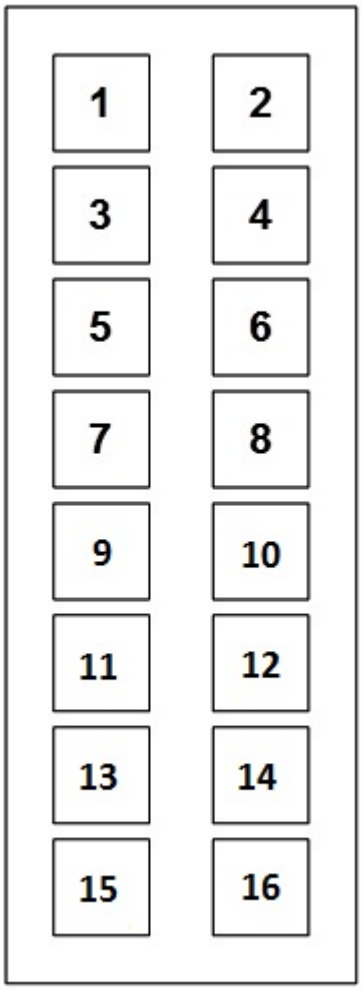
Date:_____

File Name:_____

Laser Power:_____

PMT:_____

Well No.	Sample Name	Dilution factor
1	CNTRL	
2	Std7	
3	Std6	
4	Std5	
5	Std4	
6	Std3	
7	Std2	
8	Std1	
9		
10		
11		
12		
13		
14		
15		
16		



XVI. How to Choose a Quantibody® Array?

Species-based selection:

Human (QAH-)	Mouse (QAM-)	Rat (QAR-)	Bovine (QAB-)	Canine (QAC-)
Equine (QAE-)	Feline (QAF-)	Primates (QAN-)	Porcine (QAP-)	Rabbit (QAL-)

Function-based selection:

Adhesion Molecule Arrays	Angiogenesis Arrays	Bone Metabolism Arrays	Chemokine Arrays
Custom Arrays	Cytokine Arrays	Growth Factor Arrays	IGF Signaling Arrays
IL-1 Family Arrays	Immune Response Arrays	Inflammation Arrays	Interleukin Arrays
Isotyping Arrays	MMP Arrays	Obesity Arrays	Ophthalmic Arrays
Periodontal Disease Arrays	Receptor Arrays	Th1/Th2/Th17 Arrays	

Cytokine Number-based selection:
Arrays are available in the Quantibody® platform to detect 1000 human, 640 mouse, or 67 rat proteins. GLP-Compliant testing services are also available.

To learn more about the Quantibody® Antibody Array, visit www.RayBiotech.com/Quantibody-Multiplex-Elisa-Array/

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