Quantibody® Human Cytokine Antibody Array 9000

A combination of 10 non-overlapping arrays to quantitatively measure 400 human cytokines

Catalog #: QAH-CAA-9000

User Manual
Last revised March 4, 2016

Caution:
Extraordinarily useful information enclosed

RayBiotech
The protein array pioneer

ISO 13485 Certified

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (400)	Arrays Included: QAH-INF-3 (40); QAH-GF-1 (40); QAH-CHE-1 (40); QAH-REC-1 (40); QAH-CYT-4 (40); QAH-CYT-5 (40); QAH-CYT-6 (40); QAH-CYT-7 (40); QAH-CYT-8 (40); QAH-CYT-9 (40) *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*.

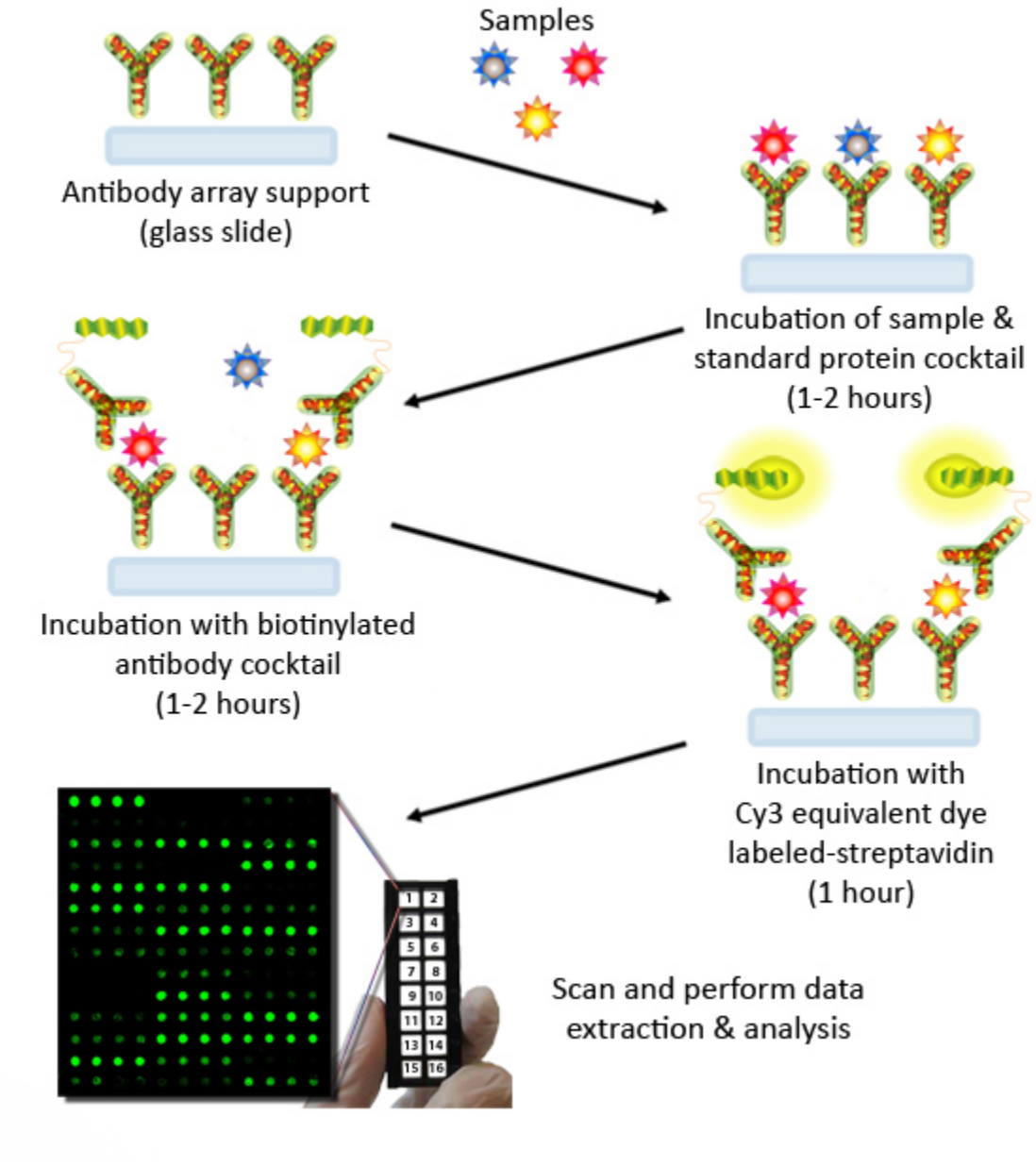
The Quantibody® array, our multiplexed sandwich ELISA-based quantitative array platform, enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines

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 660 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.
- We recommend the following parameters for your samples: 50 to 100 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

  If you experience high background or if the fluorescent signal intensities exceed the detection range, further dilution of your sample is recommended.

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.
- The Quantibody slides do not have bar codes. To help distinguish one slide from another, transcribe the slide serial number from the slide bag to the back of the slide with an ultra-fine point permanent marker. **Please Note:**Red permanent marker can significantly interfere with fluorescent signal detection. We recommend marking your slides with a green, blue or black ultra-fine point permanent marker. Please write the number on the very bottom edge of the slide. Do not write on the arrayed well areas.

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.

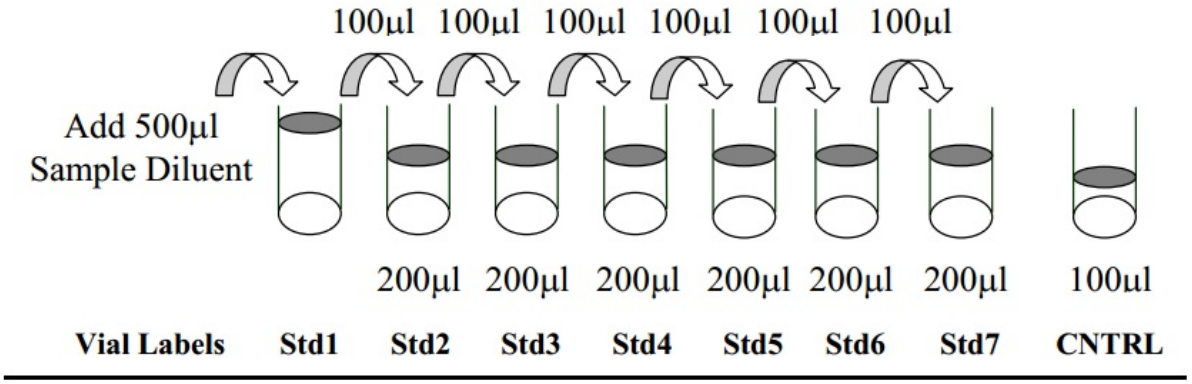
A. Completely Air Dry The Glass Slide

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 50-500 µg/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

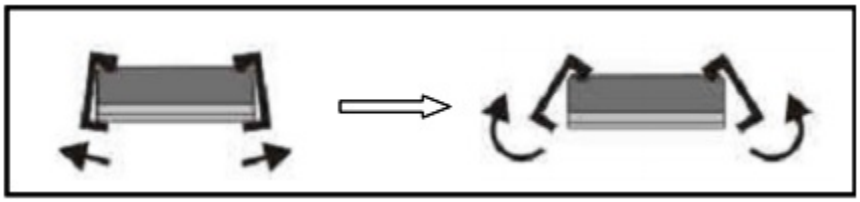
12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

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.

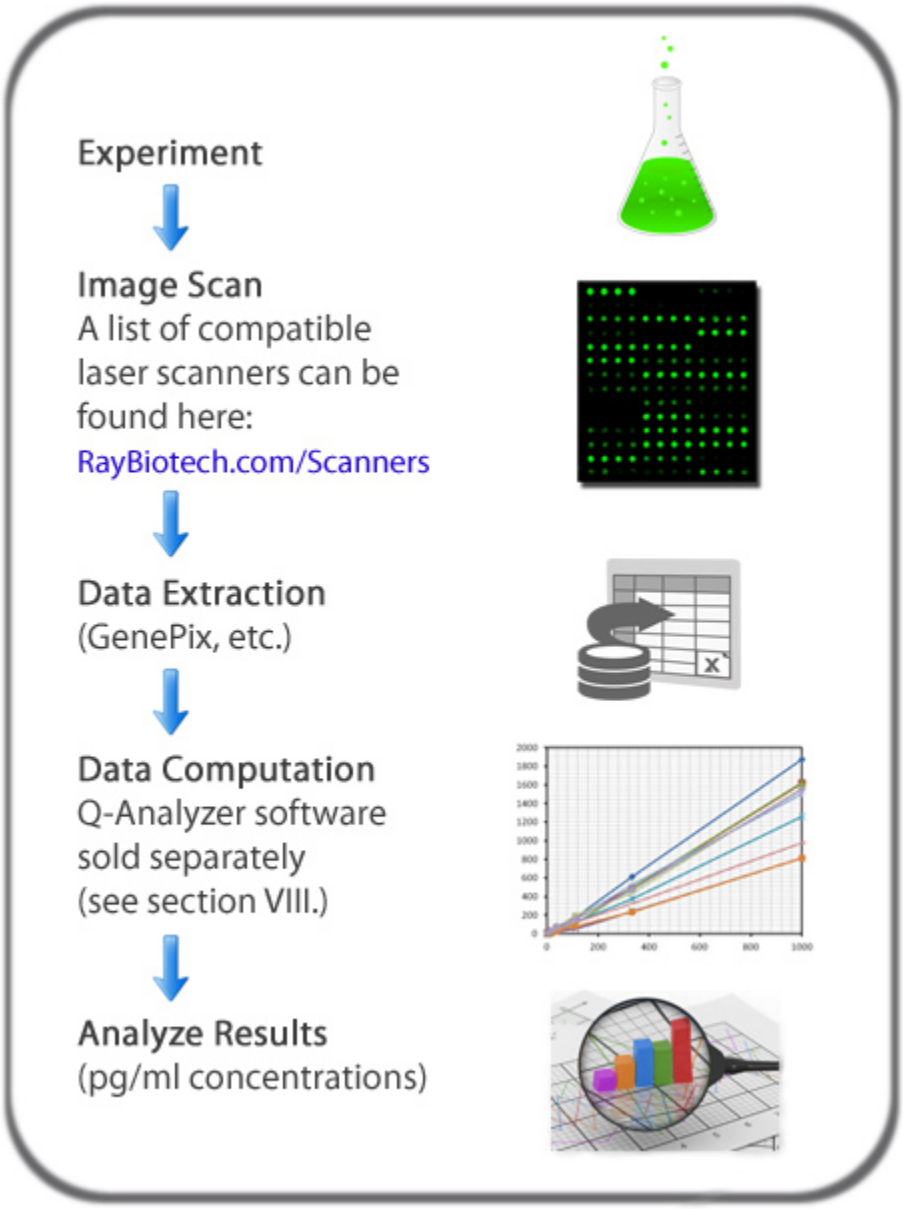
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains unsaturated.

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 along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). GAL files can be found here: www.RayBiotech.com/Gal-Files.html.

    Need help analyzing all that data? Copy and paste your data into the Q-Analyzer Tool specific for this array, catalog number: **QAH-CAA-9000-SW**. More information can be found in Section XII.



IX. Array Map & Standard Curves

Please view the individual array manuals for representative standard curve images

QAH-INF-3

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				BLC (CXCL13)			
B	Eotaxin-1 (CCL11)				Eotaxin-2 (MPIF-2)				GCSF			
C	GM-CSF				I-309 (TCA-3/CCL1)				ICAM-1 (CD54)			
D	IFN-gamma				IL-1 alpha				IL-1 beta			
E	IL-1 ra (IL-1 F3)				IL-2				IL-4			
F	IL-5				IL-6				IL-6 R			
G	IL-7				IL-8 (CXCL8)				IL-10			
H	IL-11				IL-12 p40				IL-12 p70			
I	IL-13				IL-15				IL-16			
J	IL-17A				MCP-1 (CCL2)				M-CSF			
K	MIG (CXCL9)				MIP-1 alpha (CCL3)				MIP-1 beta (CCL4)			
L	MIP-1 delta (CCL15)				PDGF-BB				RANTES (CCL5)			
M	TIMP-1				TIMP-2				TNF-alpha			
N	TNF beta				TNF RI				TNF RII			

QAH-GF-1

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Amphiregulin			
B	BDNF				bFGF				BMP-4			
C	BMP-5				BMP-7				beta-NGF			
D	EGF				EGFR				EG-VEGF (PK1)			
E	FGF-4				FGF-7 (KGF)				GDF-15			
F	GDNF				Growth Hormone				HB-EGF			
G	HGF				IGFBP-1				IGFBP-2			
H	IGFBP-3				IGFBP-4				IGFBP-6			
I	IGF-1				Insulin				M-CSF R			
J	NGFR (TNFRSF16)				NT-3				NT-4			
K	Osteoprotegerin				PDGF-AA				PLGF			
L	SCF				SCF R (CD117)				TGF alpha			
M	TGF beta 1				TGF beta 3				VEGF-A			
N	VEGFR2				VEGFR3				VEGF-D			

QAH-CHE-1

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				6Ckine (CCL21)			
B	Axl				Betacellulin(BTC)				CCL28 (MEC)			
C	CTACK (CCL27)				CXCL16				ENA-78 (CXCL5)			
D	Eotaxin-3 (CCL26)				GCP-2 (CXCL6)				GRO			
E	HCC-1 (CCL14)				HCC-4 (CCL16)				IL-9			
F	IL-17F				IL-18 BP alpha				IL-28A			
G	IL-29				IL-31				IP-10 (CXCL10)			
H	I-TAC (CXCL11)				LIF				LIGHT (TNFSF14)			
I	Lymphotactin				MCP-2 (CCL8)				MCP-3 (CCL7)			
J	MCP-4 (CCL13)				MDC (CCL22)				MIF			
K	MIP-3 alpha				MIP-3 beta				MPIF-1 (CCL23)			
L	MSP alpha/beta				NAP-2 (CXCL7)				Osteopontin			
M	PARC (CCL18)				Platelet Factor 4				SDF-1 alpha			
N	TARC (CCL17)				TECK (CCL25)				TSLP			

QAH-REC-1

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	ALCAM (CD166)				CD80 (B7-1)				BCAM (TNFRSF17)			
C	CD14				CD30 (TNFRSF21)				CD40 Ligand			
D	CEACAM-1				DR6 (TNFRSF21)				Dtk			
E	Endoglin (CD105)				ErbB3				E-Selectin			
F	Fas (Apo-1)				Flt-3 Ligand				GITR (TNFRSF18)			
G	HVEM (TNFRSF14)				ICAM-3 (CD50)				IL-1 R4 (ST2)			
H	IL-1 R1				IL-2 R gamma				IL-10 R beta			
I	IL-17 RA				IL-21 R				LIMPII			
J	Lipocalin-2 (NGAL)				L-Selectin (CD62L)				LYVE-1			
K	MICA				MICB				NRG1-beta 1			
L	PDGF R beta				PECAM-1 (CD31)				RAGE			
M	TIM-1 (KIM-1)				TRAIL R3				Trappin-2			
N	uPAR				VCAM-1				XEDAR			

QAH-CYT-4

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin A			
B	AgRP				Angiogenin				Angiopoietin-1			
C	Angiostatin				Cathepsin S				CD40			
D	Cripto-1				DAN				DKK-1			
E	E-Cadherin				TROP1 (EpCam)				Fas Ligand (TNFSF6)			
F	Fc gamma RIIB/C				Follistatin				Galectin-7			
G	ICAM-2 (CD102)				IL-13 R1				IL-13 R alpha 2			
H	IL-17B				IL-2 R alpha				IL-2 R beta			
I	IL-23				LAP/TGF beta 1				NrCAM			
J	PAI-1				PDGF-AB				Resistin			
K	SDF-1 beta				gp130				Shh-N			
L	Siglec-5 (CD170)				IL-1 R4 (ST2)				TGF beta2			
M	Tie-2				Thrombopoietin (TPO)				TRAIL R4			
N	TREM-1				VEGF-C				VEGFR1			

QAH-CYT-5

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Adiponectin (ACRP30)			
B	Adipsin				Alpha-fetoprotein				ANGPTL4			
C	Beta-2 Microglobulin				BCAM				CA125			
D	CA15-3				CEA				CRP			
E	ErbB2				Ferritin				FSH			
F	GRO alpha (CXCL1)				HCG beta				IGF-1 R			
G	IL-1 RII				IL-3				IL-18 R beta (AcPL)			
H	IL-21				Leptin				MMP-1			
I	MMP-2				MMP-3				MMP-8			
J	MMP-9				MMP-10				MMP-13			
K	NCAM-1 (CD56)				Nidogen-1				NSE			
L	Oncostatin M				Procalcitonin				Prolactin			
M	PSA-free				Siglec-9				TACE			
N	Thyroglobulin				TIMP-4				TSH			

QAH-CYT-6

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				2B4 (CD244)			
B	ADAM-9				Angiopoietin-2				APRIL			
C	BMP-2				BMP-9				C5a			
D	Cathepsin L				CD200				CD97			
E	Chemerin				DcR3				FABP2			
F	FAP				FGF-19				Galectin-3			
G	HGFR				IFN alpha/beta R2				IGF-2			
H	IGF-2 R				IL-1 R6 (IL-1 Rrp2)				IL-24			
I	IL-33 (IL-1 F11)				Kallikrein 14				Legumain			
J	LOX-1				MBL				Neprilysin			
K	Notch-1				NOV(CCN3)				Osteoactivin			
L	PD-1				PGRPs				SERPINA4			
M	sFRP-3				Thrombomodulin				TLR2			
N	TRAIL R1				Transferrin				WIF-1			

QAH-CYT-7

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ACE-2			
B	Albumin				AMICA				Angiopoietin-4			
C	BAFF				CA19-9				CD163			
D	Clusterin				CRTAM				CXCL14 (BRAK)			
E	Cystatin C				Decorin				Dkk-3			
F	DLL1				Fetuin A				aFGF (FGF-1)			
G	FOLR1				Furin				GASP-1			
H	GASP-2				G-CSF R (CD114)				HAI-2			
I	IL-17 RB				IL-27				LAG-3			
J	LDL R				Pepsinogen 1				RANK			
K	RBP4				SOST				Syndecan-1			
L	TACI				TFPI				Thrombospondin 1			
M	TRAIL R2				TRANCE				Troponin I			
N	uPA				VE-Cadherin (CDH5)				WISP-1 (CCN4)			

QAH-CYT-8

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ANGPTL3			
B	bIG-H3				CA9				Cathepsin B			
C	CD23				CHI3L1				CTLA4			
D	Dkk-4				DPPIV				EDA-A2			
E	Epo R				FGF-6				FGF-9			
F	Gas1				IGFBP-5				IL-1F5			
G	IL-1F6				IL-1F7				IL-1F8			
H	IL-1F9				IL-1F10				IL-1R5			
I	IL-17C				IL-18				IL-20			
J	IL-34				IL-5Ra				IL-10 Ra			
K	Layilin				Leptin R				Marapsin			
L	Mer				MMP-7				P-Cadherin			
M	Prostasin				PSMA				SIGIRR			
N	TGFb R3				Factor 3 (TF)				TWEAK			

QAH-CYT-9

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAMTS-13			
B	Aggrecan				Angiotensinogen				B7-H1 (CD274)			
C	BMPR-IA (ALK-3)				BMPR-II				Cadherin-11			
D	CD27 (TNFRSF7)				CD6				Ck beta 8-1 (CCL23)			
E	CNTF				DNAM-1 (CD226)				CD147 (EMMPRIN)			
F	FLRG				Follistatin-like 1				Fractalkine (CX3CL1)			
G	Galectin-1				GITR Ligand				Granulysin (LAG-2)			
H	IL-1 R3 (IL-1 R Acp)				IL-15 R alpha				IL-17E (IL-25)			
I	IL-32 alpha				CHL-1 (L1CAM-2)				LRIG3			
J	LRP-6				MEPE (OF45)				Nectin-4			
K	Periostin				Persephin				Renin			
L	RGM-B				ROBO3				S100 A8			
M	Siglec-7 (CD328)				Syndecan-3				Thrombospondin 2			
N	Thrombospondin 5				Tie-1				ULBP-2			

X. Standard Concentrations

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

QAH-INF-3	(pg/ml)	QAH-GF-1	(pg/ml)	QAH-CHE-1	(pg/ml)	QAH-REC-1	(pg/ml)	QAH-CYT-4	(pg/ml)
BLC	2,000	AR	10,000	6Ckine	40,000	4-1BB	10,000	Activin A	100,000
Eotaxin	4,000	BDNF	2,000	Axl	4,000	ALCAM	10,000	AgRP	10,000
Eotaxin-2	1,000	bFGF	20,000	BTC	20,000	B7-1	10,000	ANG	2,000
G-CSF	20,000	BMP-4	100,000	CCL28	40,000	BCMA	20,000	ANG-1	40,000
GM-CSF	1,000	BMP-5	100,000	CTACK	50,000	CD14	10,000	Angiostatin	1,000,000
I-309	4,000	BMP-7	40,000	CXCL16	20,000	CD30	10,000	Catheprin S	10,000
ICAM-1	100,000	b-NGF	10,000	ENA-78	10,000	CD40 L	10,000	CD 40	10,000
IFNg	2,000	EGF	200	Eotaxin-3	20,000	CEACAM-1	10,000	Cripto-1	10,000
IL-1a	2,000	EGF R	10,000	GCP-2	10,000	DR6	4,000	DAN	40,000
IL-1b	1,000	EG-VEGF	10,000	GRO	1,000	Dtk	20,000	DKK-1	80,000
IL-1ra	2,000	FGF-4	100,000	HCC-1	4,000	Endoglin	4,000	E-Cadherin	80,000
IL-2	2,000	FGF-7	10,000	HCC-4	10,000	ErbB3	20,000	EpCAM	20,000
IL-4	2,000	GDF-15	2,000	IL-9	200,000	E-Selectin	40,000	FAS L	2,000
IL-5	4,000	GDNF	4,000	IL-17F	100,000	Fas	2,000	Fcr RIIBC	10,000
IL-6	2,000	GH	10,000	IL-18 BPa	60,000	Flt-3L	2,000	Follistatin	40,000
IL-6sR	10,000	HB-EGF	10,000	IL-28A	10,000	GITR	10,000	Galectin-7	100,000
IL-7	4,000	HGF	4,000	IL-29	100,000	HVEM	40,000	ICAM-2	100,000
IL-8	500	IGFBP-1	5,000	IL-31	40,000	ICAM-3	100,000	IL-13 R1	10,000
IL-10	4,000	IGFBP-2	20,000	IP-10	10,000	IL-1 R4	4,000	IL-13 R2	20,000
IL-11	20,000	IGFBP-3	200,000	I-TAC	10,000	IL-1 RI	4,000	IL-17B	40,000
IL-12p40	10,000	IGFBP-4	200,000	LIF	13,000	IL-2 Rg	10,000	IL-2 Ra	10,000
IL-12p70	500	IGFBP-6	100,000	LIGHT	10,000	IL-10 Rb	4,000	IL-2 Rb	100,000
IL-13	1,000	IGF-I	20,000	Lymphotactin	100,000	IL-17R	10,000	IL-23	40,000
IL-15	4,000	Insulin	20,000	MCP-2	2,000	IL-21R	20,000	LAP	4,000
IL-16	5,000	MCSF R	40,000	MCP-3	4,000	LIMPII	4,000	NrCAM	20,000
IL-17	4,000	NGF R	10,000	MCP-4	10,000	Lipocalin-2	1,000	PAI-I	40,000
MCP-1	2,000	NT-3	40,000	MDC	10,000	L-Selectin	100,000	PDGF-AB	10,000
MCSF	4,000	NT-4	10,000	MIF	4,000	LYVE-1	2,000	Resistin	20,000
MIG	5,000	OPG	4,000	MIP-3a	4,000	MICA	10,000	SDF-1b	40,000
MIP-1a	10,000	PDGF-AA	10,000	MIP-3b	20,000	MICB	15,000	sgp130	80,000
MIP-1b	1,000	PIGF	4,000	MPIF-1	10,000	NRG1-b1	15,000	Shh N	40,000
MIP-1d	10,000	SCF	10,000	MSPa	100,000	PDGF Rb	100,000	Siglec-5	10,000
PDGF-BB	2,000	SCF R	20,000	NAP-2	4,000	PECAM-1	20,000	ST2	4,000
RANTES	20,000	TGFa	10,000	OPN	100,000	RAGE	10,000	TGF-b2	40,000
TIMP-1	40,000	TGFb1	100,000	PARC	4,000	TIM-1	10,000	Tie-2	10,000
TIMP-2	40,000	TGFb3	40,000	PF4	100,000	TRAIL R3	5,000	TPO	200,000
TNFa	2,000	VEGF	10,000	SDF-1a	10,000	Trappin-2	10,000	TRAIL R4	8,000
TNFb	20,000	VEGF R2	10,000	TARC	10,000	uPAR	40,000	TREM-1	20,000
TNF RI	40,000	VEGF R3	40,000	TECK	100,000	VCAM-1	200,000	VEGF-C	20,000
TNF RII	40,000	VEGF-D	20,000	TSLP	10,000	XEDAR	10,000	VEGF R1	40,000

QAH-CYT-5	(pg/ml)	QAH-CYT-6	(pg/ml)	QAH-CYT-7	(pg/ml)	QAH-CYT-8	(pg/ml)	QAH-CYT-9	(pg/ml)
Adiponectin	100,000	2B4	10,000	ACE-2	400,000	ANGPTL3	10,000	ADAMTS13	100,000
Adipsin	20,000	ADAM-9	100,000	Albumin	20,000	bIG-H3	10,000	Aggrecan	20,000
AFP	10,000	ANG-2	20,000	AMICA	20,000	CA9	10,000	Angiotensinogen	100,000
ANGPTL4	400,000	APRIL	200,000	ANG-4	20,000	Cathepsin B	10,000	B7-H1	10,000
B2M	10,000	BMP-2	100,000	BAFF	10,000	CD23	10,000	BMPR-IA	100,000
BCAM	40,000	BMP-9	4,000	CA19-9	100,000	CHI3L1	10,000	BMPR-II	100,000
CA125	100,000	C5a	10,000	CD163	200,000	CTLA4	4,000	Cadherin-11	400,000
CA15-3	30,000	Cathepsin L	10,000	Clusterin	10,000	Dkk-4	100,000	CD27	10,000
CEA	20,000	CD200	100,000	CRTAM	4,000	DPPIV	200,000	CD6	100,000
CRP	10,000	CD97	100,000	CXCL14	100,000	EDA-A2	10,000	Ck beta 8-1	100,000
ErbB2	10,000	Chemerin	200,000	Cystatin C	100,000	Epo R	40,000	CNTF	100,000
Ferritin	800,000	DcR3	200,000	Decorin	2,000	FGF-6	10,000	DNAM-1	100,000
FSH	10,000	FABP2	100,000	Dkk-3	100,000	FGF-9	4,000	EMMPRIN	2,000
GROa	100,000	FAP	20,000	DLL1	20,000	Gas1	100,000	FLRG	10,000
hCGb	20,000	FGF-19	20,000	Fetuin A	100,000	IGFBP-5	200,000	Follistatin-like 1	400,000
IGF-I SR	100,000	Galectin-3	4,000	aFGF	200,000	IL-1F5	200,000	Fractalkine	40,000
IL-1 sRII	20,000	HGF R	4,000	FOLR1	100,000	IL-1F6	200,000	Galectin-1	20,000
IL-3	10,000	IFNab R2	100,000	Furin	200,000	IL-1F7	100,000	GITR Ligand	200,000
IL-18 Rb	20,000	IGF-II	100,000	GASP-1	2,000	IL-1F8	4,000	Granulysin	4,000
IL-21	100,000	IGF-II R	20,000	GASP-2	100,000	IL-1F9	100,000	IL-1 R3	10,000
Leptin	40,000	IL-1 R6	100,000	G-CSF R	10,000	IL-1F10	200,000	IL-15 R	2,000
MMP-1	40,000	IL-24	100,000	HAI-2	40,000	IL-1R5	1,000	IL-17E	40,000
MMP-2	100,000	IL-33	10,000	IL-17B R	100,000	IL-17C	400,000	IL-32 alpha	4,000
MMP-3	40,000	Kallikrein 14	4,000	IL-27	10,000	IL-18	40,000	L1CAM-2	200,000
MMP-8	10,000	Legumain	10,000	LAG-3	100,000	IL-20	100,000	LRIG3	200,000
MMP-9	20,000	LOX-1	2,000	LDL R	2,000	IL-34	40,000	LRP-6	200,000
MMP-10	10,000	MBL	1,000	Pepsinogen I	20,000	IL-5 Ra	400,000	MEPE	200,000
MMP-13	10,000	Neprilysin	20,000	RANK	100,000	IL-10 Ra	200,000	Nectin-4	20,000
NCAM-1	200,000	Notch-1	4,000	RBP4	20,000	Layilin	10,000	Periostin	200,000
Nidogen-1	20,000	NOV	4,000	SOST	40,000	Leptin R	100,000	Persephin	100,000
NSE	100,000	Osteoactivin	10,000	Syndecan-1	100,000	Marapsin	20,000	Renin	10,000
OSM	10,000	PD-1	4,000	TACI	40,000	Mer	10,000	RGM-B	100,000
Procalcitonin	100,000	PGRP-5	1,000	TFPI	100,000	MMP-7	100,000	ROBO3	2,000
Prolactin	400,000	Serpin A4	10,000	TSP-1	100,000	P-Cadherin	100,000	S100A8	10,000
PSA	20,000	sFRP-3	100,000	TRAIL R2	4,000	Prostasin	20,000	Siglec-7	2,000
Siglec-9	40,000	Thrombomodulin	100,000	TRANCE	40,000	PSMA	100,000	Syndecan-3	100,000
TACE	100,000	TLR2	20,000	Troponin I	200,000	SIGIRR	100,000	Thrombospondin-2	10,000
Thyroglobulin	100,000	TRAIL R1	10,000	uPA	4,000	TGFb RIII	20,000	Thrombospondin-5	10,000
TIMP-4	20,000	Transferrin	100,000	VE-Cadherin	200,000	TF	4,000	Tie-1	10,000
TSH	20,000	WIF-1	20,000	WISP-1	200,000	TWEAK	100,000	ULBP-2	4,000

XI. Spiking & Recovery

Please view the individual array manuals for spiking & recovery data

XII. Quantibody® Q-Analyzer

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).

The Q-Analyzer Tool specific for this array is catalog number: **QAH-CAA-9000-SW**.

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. Publications Citing This Product

1. Mao Y., Yen H., Sun Y., Lv Z., Huang R. Development of non-overlapping multiplex antibody arrays for the quantitative measurement of 400 human and 200 mouse proteins in parallel (TECH1P.849). The Journal of ImmunologyMay 1, 2014vol. 192 no. 1 Supplement 69.17
   Species: Human
   Sample Type: Serum

2. Mao Y., Yen H., Sun Y., Lv Z., Huang R. Development of non-overlapping multiplex antibody arrays for the quantitative measurement of 400 human and 200 mouse proteins in parallel (TECH1P.849). The Journal of ImmunologyMay 1, 2014vol. 192 no. 1 Supplement 69.17
   Species: Human
   Sample Type: Plasma

More citations for this product may be available.
Contact techsupport@raybiotech.com.

Note: The citations listed above are for the use of this combination array. Citations for the individual arrays can be found in the individual array manuals.

XV. Experiment Record Form

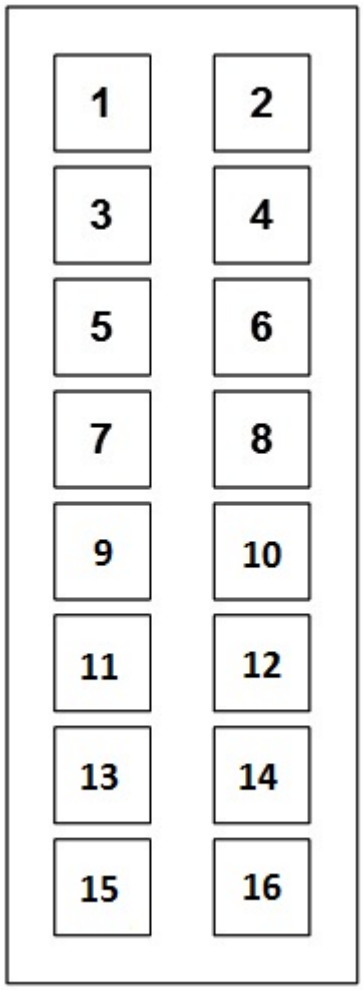
Date:_____

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 660 human, 200 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.html