Quantibody® Mouse Cytokine Antibody Array 400

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

Catalog #: QAM-CAA-400

User Manual
Last revised July 18, 2019

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 (400)	Arrays Included: QAM-CYT-4 (40); QAM-CYT-5 (40); QAM-CYT-6 (40); QAM-CYT-7 (40); QAM-CYT-8 (40); QAM-CYT-9 (40); QAM-CYT-10 (40); QAM-CYT-11 (40); QAM-CYT-12 (40); QAM-CYT-13 (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.

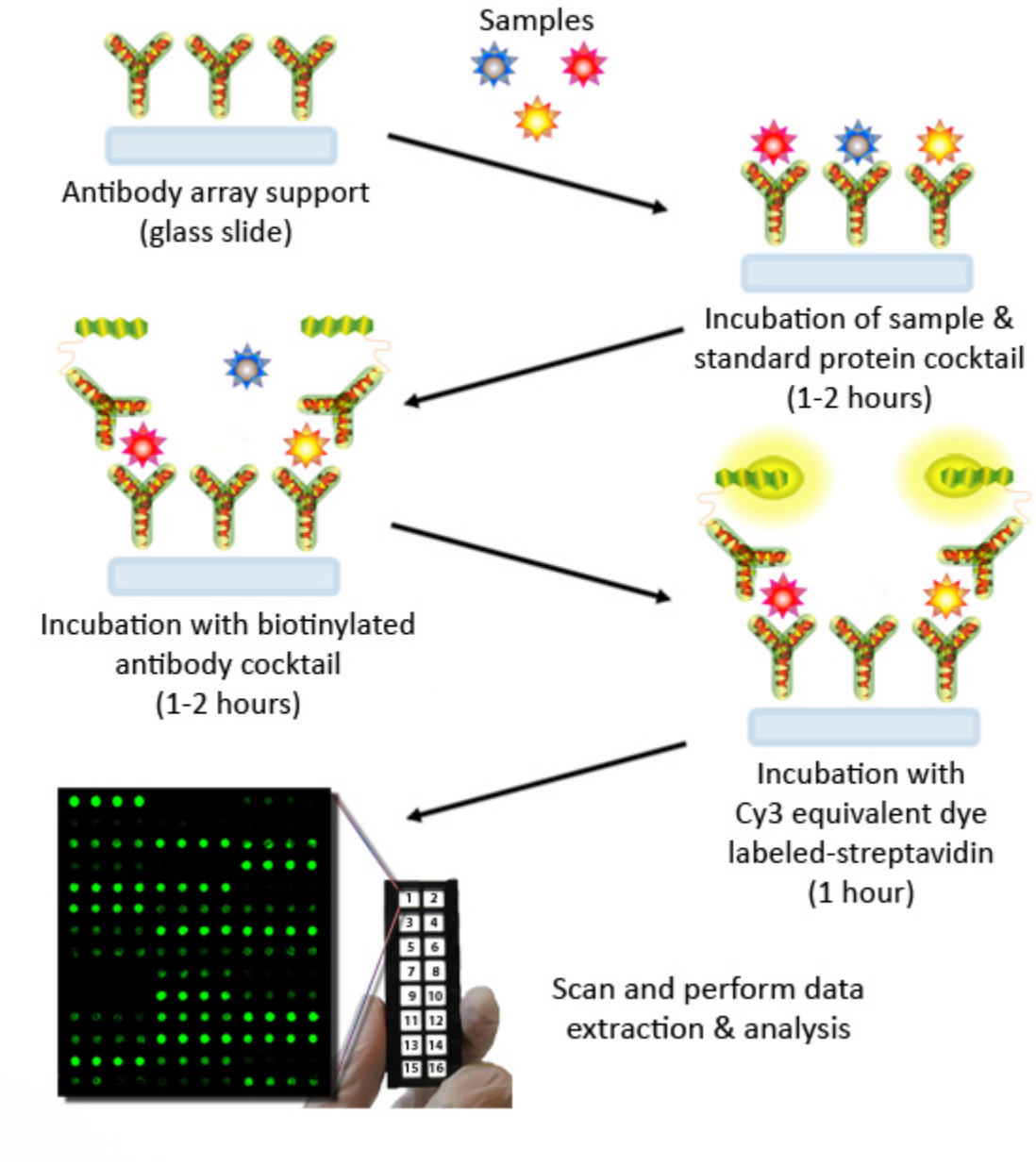
The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent assay (ELISA). In this method, target protein is immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the 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 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

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.

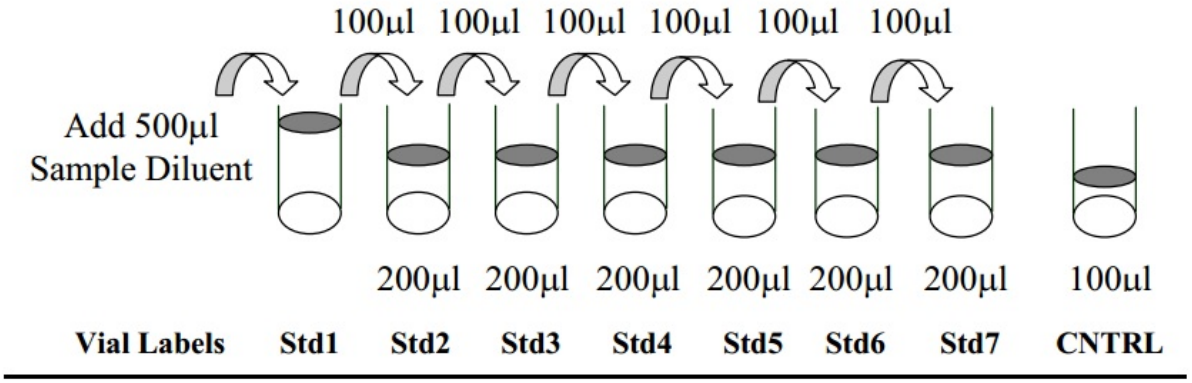
A. Completely Air Dry The Glass Slide

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

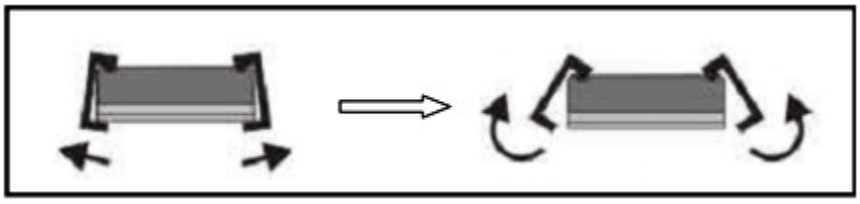
12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

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.

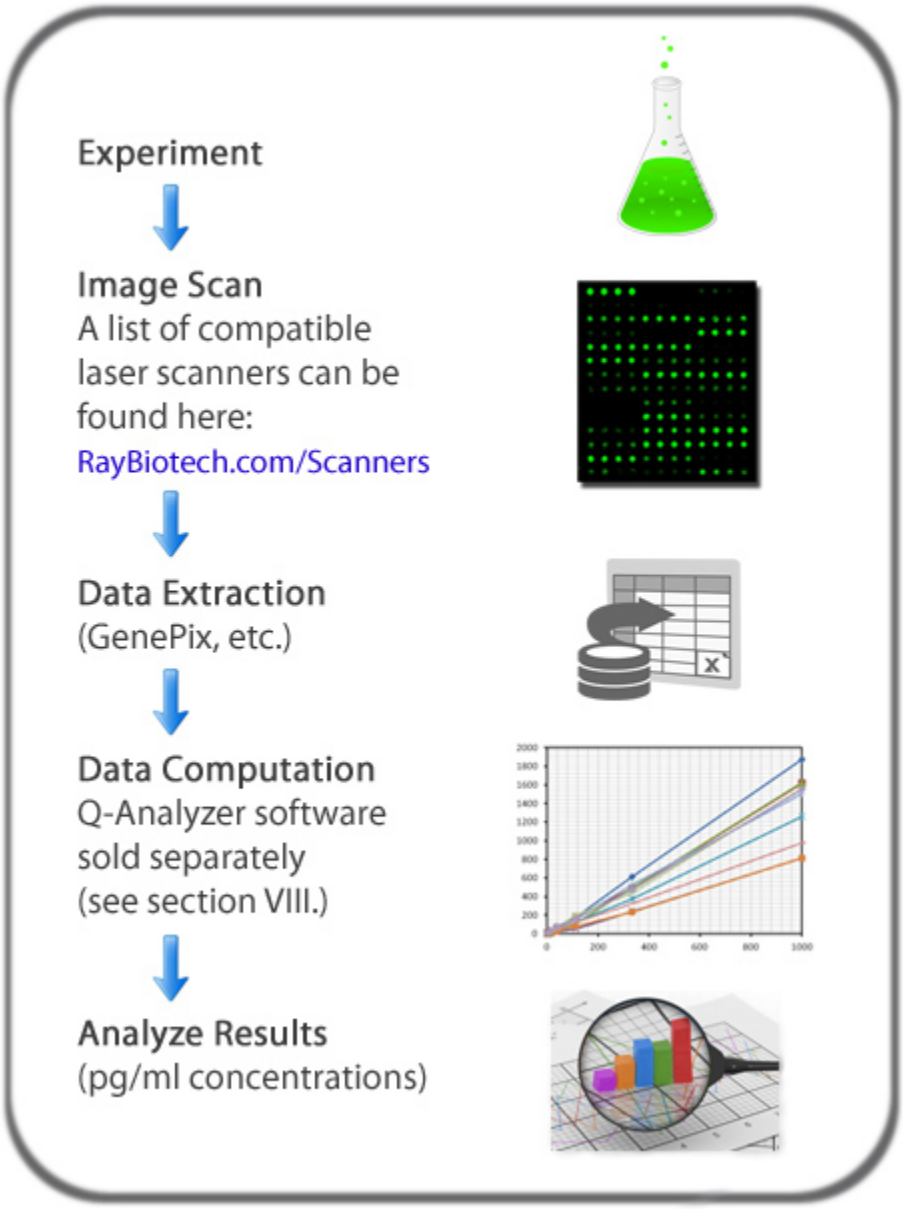
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains 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 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: **QAM-CAA-400-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

QAM-CYT-4

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Amphiregulin			
B	Axl				CD27 Ligand				CD30 (TNFRSF8)			
C	CD40 (TNFRSF5)				CXCL16				EGF			
D	E-Selectin				Fractalkine				GITR (TNFRSF18)			
E	HGF				IGFBP-2				IGFBP-3			
F	IGFBP-5				IGFBP-6				IGF-1			
G	IL-12 p70				IL-17E (IL-25)				IL-17F			
H	IL-1 ra (IL-1 F3)				IL-2 R alpha				IL-20			
I	IL-23 p19				IL-28A				I-TAC (CXCL11)			
J	MDC (CCL22)				MIP-2				MIP-3 alpha (CCL20)			
K	Osteopontin (SPP1)				Osteoprotegerin				Prolactin			
L	Pro-MMP-9				P-Selectin				Resistin			
M	SCF				SDF-1 alpha				Thrombopoietin (TPO)			
N	VCAM-1 (CD106)				VEGF-A				VEGF-D			

QAM-CYT-5

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				bFGF			
B	BLC (CXCL13)				CD30 Ligand				Eotaxin-1 (CCL11)			
C	Eotaxin-2 (MPIF-2)				Fas Ligand (TNFSF6)				GCSF			
D	GM-CSF				ICAM-1 (CD54)				IFN-gamma			
E	IL-1 alpha				IL-1 beta				IL-2			
F	IL-3				IL-4				IL-5			
G	IL-6				IL-7				IL-10			
H	IL-12 p40				IL-13				IL-15			
I	IL-17A				IL-21				KC (CXCL1)			
J	Leptin				LIX				MCP-1 (CCL2)			
K	MCP-5				M-CSF				MIG (CXCL9)			
L	MIP-1 alpha (CCL3)				MIP-1 gamma				Platelet Factor 4			
M	RANTES (CCL5)				TARC (CCL17)				I-309 (CCL1)			
N	TNF RI (TNFRSF1A)				TNF RII (TNFRSF1B)				TNF-alpha			

QAM-CYT-6

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	**Ace**				**ALK-1**				**Cardiotriohin-1 (CT-1)**			
C	**CD27 (TNFRSF7)**				**CD40 Ligand**				**CTLA-4 (CD152)**			
D	**Decorin**				**DKK-1**				**Dtk**			
E	**Endoglin (CD105)**				**Fc-gamma-RIIB**				**Flt-3 Ligand**			
F	**Galectin-1**				**Galectin-3**				**Gas 1**			
G	**Gas 6**				**GITR Ligand**				**HAI-1**			
H	**HGFR**				**IL-1 R4 (ST2)**				**IL-3 R beta**			
I	**IL-9**				**JAM-A (CD321)**				**Leptin R**			
J	**L-selectin (CD62L)**				**Lymphotactin**				**MadCAM-1**			
K	**MFG-E8**				**MIP-3 beta (CCL19)**				**Neprilysin**			
L	**Pentraxin-3 (TSG-14)**				**RAGE**				**TACI**			
M	**TREM-1**				**TROY**				**TSLP**			
N	**TWEAK R**				**VEGF R1**				**VEGF R3**			

QAM-CYT-7

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				CD80 (B7-1)			
B	BAFF R				Betacellulin (BTC)				C5a			
C	CCL6				CD48 (SLAMF2)				CD6			
D	Chemerin				Clusterin				CXCL15			
E	Cystatin C				DAN				DLL4			
F	EDAR				Endocan				Fetuin A			
G	H60				IL-33				IL-7 R alpha			
H	Kremen-1				Limitin				Lipocalin-2 (NGAL)			
I	LOX-1				Marapsin				MBL-2			
J	Meteorin				Nope				NOV (CCN3)			
K	Osteoactivin				OX40 Ligand				P-Cadherin			
L	Periostin				PIGF-2				Progranulin			
M	Prostasin				Renin 1				Testican 3			
N	TIM-1 (KIM-1)				TRAIL (TNFSF10)				Tryptase epsilon			

QAM-CYT-8

Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				6Ckine			
B	Activin A				ADAMTS1				Adiponectin			
C	ANG-3				ANGPTL3				Artemin			
D	CCL28				CD36				Chordin			
E	CRP				E-Cadherin				Epigen			
F	Epiregulin				Fas				Galectin-7			
G	gp130				Granzyme B				Gremlin			
H	IFN-γ R1				IL-17B				IL-17B R			
I	IL-22				MIP-1β				MMP-2			
J	MMP-3				MMP-10				PDGF-AA			
K	Persephin				sFRP-3				Shh-N			
L	SLAM				TCK-1				TECK			
M	TGFβ1				TRANCE				TremL1			
N	TWEAK				VEGF-B				VEGF-R2			

QAM-CYT-9

(mCYT9 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				2B4			
B	4-1BB Ligand				Activin RIB				Ameloblastin			
C	ANGPTL7				B7-2				B7-H2			
D	B7-H3				B7-H4				BAFF			
E	C1q R1				Cathepsin H				CD117			
F	CD157				CD200				CD229			
G	CD28				CD300b				CD39			
H	CD44				CD45				CD69			
I	CD99-L2				CHL-1				CHRDL2			
J	COCO				CRACC				CXADR			
K	DcTRAIL R1				Dectin-2				DNAM-1			
L	DNER				Endoglycan				EphA8			
M	EphB2				EphB4				EphB6			
N	Ephrin-A2				Ephrin-A4				FGF-21			

QAM-CYT-10

(mCYT10 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAM9			
B	CD14				CD39L3				CDNF			
C	Cripto				CXCL14				Epimorphin			
D	Erythropoietin R				Flt-3				Follistatin			
E	Frizzled-1				Frizzled-4				FSTL1			
F	GDF-3				GDF-7				GFR alpha-3			
G	IFN-gamma R2				IL-1 RI				IL-1 RII			
H	IL-10 R beta				IL-11 R alpha				IL-15 R alpha			
I	IL-17 RA				IL-17 RC				IL-20 R beta			
J	IL-1F8				IL-21 R				IL-23 R			
K	IL-28A				JAM-C				Klotho beta			
L	Layilin				LDL R				LIF			
M	Matrilin-3				Lymphotoxin beta R				Nephrin			
N	Neurocan				NKp46				Laminin alpha 4			

QAM-CYT-11

(mCYT11 Map) 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	ADAM15				AFP				ASAHL			
C	C4.4A				CA12				CA14			
D	CA4				CA9				Cadherin-4			
E	CD2				CD4				CD90			
F	CDCP1				CEACAM-1				CLEC9a			
G	CFVII				Contactin-4				Contactin-6			
H	EpCAM				EphA5				FCRL1			
I	FGF-10				FGF-4				FGF-6			
J	FLRG				G-CSF R				GPV			
K	GPVI				HDAC8				HS6ST3			
L	IGF-II				IGSF8				IL-1 R6			
M	IL-1 R7				IL-16				IL-17C			
N	IL-18 BPc				IL-31				IL-34			

QAM-CYT-12

(mCYT12 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ASAM			
B	Cystatin B				DLL1				Kallikrein 7			
C	Kremen-2				LAMP1				LIGHT			
D	LIMPII				LRPAP				LRRC32			
E	Matrilin-2				Mcpt6				MEP1A			
F	MEPE				MESDC2				METRNL			
G	Mimecan				Nectin-2				Neurturin			
H	NGF R				NgR				Olfactomedin-1			
I	Oncostatin M				OSM R beta				Osteoadherin			
J	OX40				PD-1				PDGF R beta			
K	PD-L2				PILR-beta				PLA2G2A			
L	Plexin A1				Plexin C1				Podocalyxin			
M	Podoplanin				Protocadherin-12				Prss34			
N	RANK				Reg2				Relaxin-1			

QAM-CYT-13

(mCYT13 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Ret			
B	RGM-B				RGM-C				ROBO3			
C	SEMA3C				SEMA3F				SEMA4C			
D	SEMA4F				SEMA4G				SEMA6C			
E	Siglec-3				Siglec-E				SIGNR1			
F	Slit2				SMOC-1				SorCS2			
G	SP-D				SR-AI				STC-2			
H	Syndecan-1				Syndecan-3				TFPI-2			
I	TGF-beta RI				TGF-beta RII				TGM2			
J	TIGIT				TIM-3				TLR2			
K	TNFRH3				Tpo R				TRAIL R2			
L	TREM-2				TrkC				TROP-2			
M	Trypsin 3				Tryptase-5				TSLP R			
N	uPAR				VE-Cadherin				Wnt-2b			

X. Standard Concentrations

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

QAM-CYT-4	(pg/ml)	QAM-CYT-5	(pg/ml)	QAM-CYT-6	(pg/ml)	QAM-CYT-7	(pg/ml)	QAM-CYT-8	(pg/ml)
AR	2,000	bFGF	5,000	4-1BB	25,000	B7-1	4,000	6Ckine	20,000
Axl	10,000	BLC	10,000	ACE	100,000	BAFF R	1,000	Activin A	4,000
CD27L	20,000	CD30L	2,000	ALK-1	10,000	BTC	2,000	ADAMTS1	40,000
CD30T	10,000	Eotaxin	1,000	CT-1	40,000	C5a	1,000	Adiponectin	10,000
CD40	10,000	Eotaxin-2	1,000	CD27	25,000	CCL6	40,000	ANG-3	40,000
CXCL16	1,000	Fas L	10,000	CD40L	40,000	CD48	2,000	ANGPTL3	100,000
EGF	2,000	G-CSF	20,000	CTLA-4	2,500	CD6	1,000	Artemin	4,000
E-selectin	4,000	GM-CSF	10,000	Decorin	5,000	Chemerin	100,000	CCL28	100,000
Fractalkine	100,000	ICAM-1	10,000	Dkk-1	40,000	Clusterin	100,000	CD36	200,000
GITR	4,000	IFNg	4,000	Dtk	20,000	CXCL15	200,000	Chordin	10,000
HGF	20,000	IL-1a	2,000	Endoglin	10,000	Cystatin C	2,000	CRP	4,000
IGFBP-2	100,000	IL-1b	4,000	Fcg RIIB	10,000	DAN	100,000	E-Cadherin	10,000
IGFBP-3	20,000	IL-2	10,000	Flt-3L	25,000	DLL4	40,000	Epigen	20,000
IGFBP-5	40,000	IL-3	2,000	Galectin-1	10,000	EDAR	20,000	Epiregulin	200,000
IGFBP-6	40,000	IL-4	500	Galectin-3	2,000	Endocan	20,000	Fas	10,000
IGF-I	10,000	IL-5	10,000	Gas 1	2,000	Fetuin A	100,000	Galectin-7	100,000
IL-12p70	4,000	IL-6	4,000	Gas 6	2,500	H60	2,000	gp130	10,000
IL-17E	40,000	IL-7	10,000	GITR L	1,000	IL-33	4,000	Granzyme B	20,000
IL-17F	40,000	IL-10	10,000	HAI-1	10,000	IL-7 Ra	40,000	Gremlin	100,000
IL-1ra	4,000	IL-12p40	1,000	HGF R	25,000	Kremen-1	4,000	IFN-γ R1	2,000
IL-2 Ra	10,000	IL-13	20,000	IL-1 R4	40,000	Limitin	1,000	IL-17B	200,000
IL-20	20,000	IL-15	100,000	IL-3 Rb	40,000	Lipocalin-2	100,000	IL-17B R	100,000
IL-23	40,000	IL-17	4,000	IL-9	20,000	LOX-1	4,000	IL-22	40,000
IL-28	2,000	IL-21	20,000	JAM-A	5,000	Marapsin	20,000	MIP-1b	4,000
I-TAC	20,000	KC	2,000	Leptin R	5,000	MBL-2	2,000	MMP-2	20,000
MDC	1,000	Leptin	100,000	L-Selectin	10,000	Meteorin	40,000	MMP-3	10,000
MIP-2	1,000	LIX	20,000	Lymphotactin	200,000	Nope	10,000	MMP-10	1,000
MIP-3a	1,000	MCP-1	4,000	MadCAM-1	10,000	NOV	40,000	PDGF-AA	4,000
OPN	20,000	MCP-5	1,000	MFG-E8	40,000	Osteoactivin	10,000	Persephin	4,000
OPG	20,000	M-CSF	2,000	MIP-3b	1,000	OX40 Ligand	4,000	sFRP-3	20,000
Prolactin	10,000	MIG	10,000	Neprilysin	20,000	P-Cadherin	4,000	Shh-N	10,000
Pro-MMP-9	100,000	MIP-1a	10,000	Pentraxin 3	10,000	Periostin	4,000	SLAM	100,000
P-selectin	4,000	MIP-1g	1,000	RAGE	25,000	PIGF-2	1,000	TCK-1	200,000
Resistin	2,000	PF-4	20,000	TACI	50,000	Progranulin	100,000	TECK	200,000
SCF	10,000	RANTES	4,000	TREM-1	10,000	Prostasin	100,000	TGFb1	100,000
SDF-1a	100,000	TARC	4,000	TROY	4,000	Renin 1	40,000	TRANCE	40,000
TPO	100,000	TCA-3	2,000	TSLP	4,000	Testican 3	40,000	TremL1	40,000
VCAM-1	4,000	TNF RI	500	TWEAK R	25,000	TIM-1	100,000	TWEAK	20,000
VEGF	4,000	TNF RII	2,000	VEGF R1	10,000	TRAIL	10,000	VEGF-B	10,000
VEGF-D	4,000	TNFa	1,000	VEGF R3	10,000	Tryptase ε	100,000	VEGF-R2	10,000

QAM-CYT-9	(pg/ml)	QAM-CYT-10	(pg/ml)	QAM-CYT-11	(pg/ml)	QAM-CYT-12	(pg/ml)	QAM-CYT-13	(pg/ml)
2B4	20,000	ADAM9	1,000	ACE-2	10,000	ASAM	10,000	Ret	100,000
4-1BB Ligand	10,000	CD14	100,000	ADAM15	40,000	Cystatin B	200,000	RGM-B	4,000
Activin RIB	1,000	CD89L3	10,000	AFP	20,000	DLL1	2,000	RGM-C	4,000
Ameloblastin	4,000	CDNF	1,000	ASAHL	100,000	Kallikrein 7	20,000	ROBO3	4,000
ANGPTL7	20,000	Cripto	10,000	C4.4A	10,000	Kremen-2	40,000	SEMA3C	100,000
B7-2	4,000	CXCL14	8,000	CA12	10,000	LAMP1	20,000	SEMA3F	20,000
B7-H2	8,000	Epimorphin	4,000	CA14	10,000	LIGHT	40,000	SEMA4C	40,000
B7-H3	40,000	Erythropoietin R	20,000	CA4	400	LIMPII	100,000	SEMA4F	20,000
B7-H4	40,000	Flt-3	100,000	CA9	4,000	LRPAP	20,000	SEMA4G	40,000
BAFF	1,000	Follistatin	2,000	Cadherin-4	10,000	LRRC32	40,000	SEMA6C	40,000
C1q R1	100	Frizzled-1	40,000	CD2	100,000	Matrilin-2	40,000	Siglec-3	1,000
Cathepsin H	40,000	Frizzled-4	20,000	CD4	20,000	Mcpt6	400,000	Siglec-E	4,000
CD117	4,000	FSTL1	10,000	CD90	4,000	MEP1A	100,000	SIGNR1	10,000
CD157	4,000	GDF-3	40,000	CDCP1	2,000	MEPE	10,000	Slit2	100,000
CD200	4,000	GDF-7	4,000	CEACAM-1	100,000	MESDC2	1,000	SMOC-1	10,000
CD229	20,000	GFR alpha-3	10,000	CLEC9a	20,000	METRNL	100,000	SorCS2	100,000
CD28	20,000	IFN-gamma R2	400	CPVII	100,000	Mimecan	40,000	SP-D	10,000
CD300b	40,000	IL-1 RI	100,000	Contactin-4	20,000	Nectin-2	4,000	SR-AI	1,000
CD39	100,000	IL-1 RII	40,000	Contactin-6	20,000	Neurturin	10,000	STC-2	100,000
CD44	10,000	IL-10R beta	20,000	EpCAM	4,000	NGF R	1,000	Syndecan-1	10,000
CD45	20,000	IL-11 R alpha	2,000	EphA5	4,000	NgR	1,000	Syndecan-3	4,000
CD69	4,000	IL-15 R alpha	1,000	FCRL1	100,000	Olfactomedin-1	10,000	TFPI-2	100,000
CD99-L2	200	IL-17 RA	2,000	FGF-10	200,000	Oncostatin M	4,000	TGF-beta RI	10,000
CHL-1	10,000	IL-17 RC	4,000	FGF-4	10,000	OSM R beta	10,000	TGF-beta RII	10,000
CHRDL2	10,000	IL-20R beta	40,000	FGF-6	4,000	Osteoadherin	100,000	TGM2	100,000
COCO	20,000	IL-1F8	4,000	FLRG	10,000	OX40	1,000	TIGIT	100,000
CRACC	40,000	IL-21 R	100,000	G-CSF R	100,000	PD-1	10,000	TIM-3	200,000
CXADR	2,000	IL-23 R	1,000	GPV	20,000	PDGF R beta	40,000	TLR2	100,000
DcTRAIL R1	20,000	IL-28A	2,000	GPVI	4,000	PD-L2	40,000	TNFRH3	10,000
Dectin-2	400	JAM-C	400	HDAC8	20,000	PILR-beta	4,000	Tpo R	10,000
DNAM-1	1,000	Klotho beta	100,000	H6ST3	4,000	PLA2G2A	100,000	TRAIL R2	10,000
DNER	1,000	Layilin	4,000	IGF-II	1,000	Plexin A1	40,000	TREM-2	4,000
Endoglycan	10,000	LDL R	100,000	IGSF8	10,000	Plexin C1	20,000	TrkC	4,000
EphA8	10,000	LIF	100	IL-1 R6	40,000	Podocalyxin	10,000	TROP-2	20,000
EphB2	1,000	Matrilin-3	2,000	IL-1 R7	20,000	Podoplanin	1,000	Trypsin 3	4,000
EphB4	1,000	Lymphotoxin bet	200	IL-16	200	Protocadherin-12	100,000	Tryptase-5	4,000
EphB6	2,000	Nephrin	20,000	IL-17C	20,000	Prss34	200,000	TSLP R	1,000
Ephrin-A2	2,000	Neurocan	4,000	IL-18 BPc	4,000	RANK	4,000	uPAR	4,000
Ephrin-A4	200,000	NKp46	4,000	IL-31	4,000	Reg2	4,000	VE-Cadherin	100,000
FGF-21	1,000	Laminin alpha 4	100,000	IL-34	2,000	Relaxin-1	10,000	Wnt-2b	200,000

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: **QAM-CAA-400-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 Mouse and 200 mouse proteins in parallel (TECH1P.849). The Journal of ImmunologyMay 1, 2014vol. 192 no. 1 Supplement 69.17
   Species: Mouse
   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 Mouse and 200 mouse proteins in parallel (TECH1P.849). The Journal of ImmunologyMay 1, 2014vol. 192 no. 1 Supplement 69.17
   Species: Mouse
   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

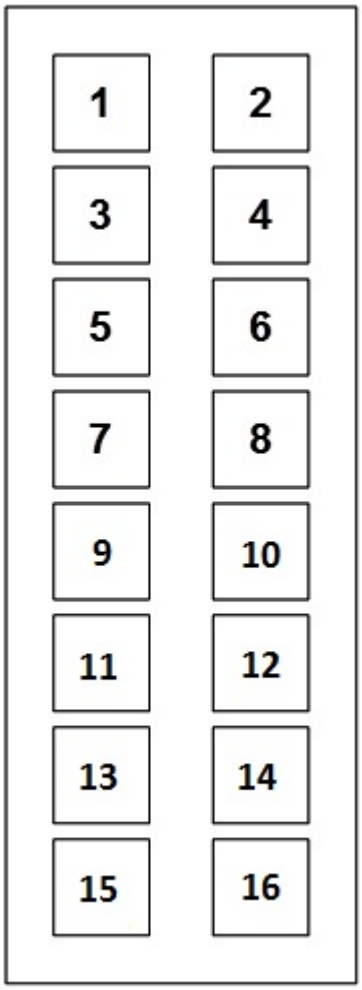
Date:_____

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 1000 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