

Quantibody[®] Human Cytokine Antibody Array 8000 --Quantitative measurement of 360 human cytokines

Patent Pending Technology

User Manual (Version Dec 2013)

Quantibody[®] Human Cytokine Antibody Array 8000

(A combination of nine forty-cytokine Quantibody arrays to quantitatively measure the concentration of 360 human cytokines)

Cat # QAH-CAA-8000

Quantibody[®] Human Inflammation Array 3 (Cat# QAH-INF-3)
Quantibody[®] Human Growth Factor Array 1 (Cat# QAH-GF-1)
Quantibody[®] Human Chemokine Array 1 (Cat# QAH-CHE-1)
Quantibody[®] Human Receptor Array 1 (Cat# QAH-REC-1)
Quantibody[®] Human Cytokine Array 4 (Cat# QAH-CYT-4)
Quantibody[®] Human Cytokine Array 5 (Cat# QAH-CYT-5)
Quantibody[®] Human Cytokine Array 6 (Cat# QAH-CYT-6)
Quantibody[®] Human Cytokine Array 7 (Cat# QAH-CYT-7)
Quantibody[®] Human Cytokine Array 8 (Cat# QAH-CYT-8)



RayBiotech, Inc.

**We Provide You With Excellent
Protein Array Systems and Service**

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Website:www.raybiotech.com Email: info@raybiotech.com**

Cytokine Detected	360
Quantibody® Human Inflammation Array 3 (40)	BLC, Eotaxin, Eotaxin-2, G-CSF, GM-CSF, I-309, ICAM-1, IFN γ , IL-1 α , IL-1 β , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-6 sR, IL-7, IL-8, IL-10, IL-11, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, MCP-1, M-CSF, MIG, MIP-1 α , MIP-1 β , MIP-1 δ , PDGF-BB, RANTES, TIMP-1, TIMP-2, TNF α , TNF β , TNF sRI, TNF sRII
Quantibody® Human Growth Factor Array 1 (40)	AR, BDNF, bFGF, BMP-4, BMP-5, BMP-7, β -NGF, EGF, EGF R, EG-VEGF, FGF-4, FGF-7, GDF-15, GDNF, GH, HB-EGF, HGF, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-6, IGF-I, Insulin, MCSF R, NGF R, NT-3, NT-4, OPG, PDGF-AA, PIGF, SCF, SCF R, TGF α , TGF β 1, TGF β 3, VEGF, VEGF R2, VEGF R3, VEGF-D
Quantibody® Human Chemokine Array 1 (40)	6Ckine, Axl, BTC, CCL28, CTACK, CXCL16, ENA-78, Eotaxin-3, GCP-2, GRO, HCC-1, HCC-4, IL-9, IL-17F, IL-18 BP α , IL-28A, IL-29, IL-31, IP-10, I-TAC, LIF, LIGHT, Lymphotactin, MCP-2, MCP-3, MCP-4, MDC, MIF, MIP-3 α , MIP-3 β , MPIF-1, MSP α , NAP-2, OPN, PARC, PF4, SDF-1 α , TARC, TECK, TSLP
Quantibody® Human Receptor Array 1 (40)	4-1BB, ALCAM, B7-1, BCMA, CD14, CD30, CD40L, CEACAM-1, DR6, Dtk, Endoglin, ErbB3, E-Selectin, Fas, Flt-3L, GITR, HVEM, ICAM-3, IL-1 R4, IL-1 RI, IL-10 R β , IL-17R, IL-2R γ , IL-21R, LIMP2, Lipocalin-2, L-Selectin, LYVE-1, MICA, MICB, NRG1- β 1, PDGF R β , PECAM-1, RAGE, TIM-1, TRAIL R3, Trappin-2, uPAR, VCAM-1, XEDAR
Quantibody® Human Cytokine Array 4 (40)	Activin A, AgRP, Angiogenin, ANG-1, Angiostatin, Catheprin S, CD40, Cripto-1, DAN, DKK-1, E-Cadherin, EpCAM, Fas L, Fc γ RIIB/C, Follistatin, Galectin-7, ICAM-2, IL-13 R1, IL-13R2, IL-17B, IL-2 R α , IL-2 R β , IL-23, LAP, NrCAM, PAI-1, PDGF-AB, Resistin, SDF-1 β , sgp130, Shh N, Siglec-5, ST2, TGF- β 2, Tie-2, TPO, TRAIL R4, TREM-1, VEGF-C, VEGF R1
Quantibody® Human Cytokine Array 5 (40)	Adiponectin, Adipsin, AFP, ANGPTL4, B2M, BCAM, CA125, CA15-3, CEA, CRP, ErbB2, Ferritin, FSH, GRO α , HCG β , IGF-1 SR, IL-1 sRII, IL-3, IL-18 R β , IL-21, Leptin, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-10, MMP-13, NCAM-1, Nidogen-1, NSE, OSM, TSH, Procalcitonin, Prolactin, PSA, Siglec-9, TACE, Thyroglobulin, TIMP-4
Quantibody® Human Cytokine Array 6 (40)	2B4, ADAM-9, ANG-2, APRIL, BMP-2, BMP-9, C5a, Cathepsin L, CD200, CD97, Chemerin, DcR3, FABP2, FAP, FGF-19, Galectin-3, HGF R, IFN α / β R2, IGF-II, IGF-II R, IL-1R6, IL-24, IL-33, Kallikrein 14, Legumain, LOX-1, MBL, Nephrilysin, Notch-1, NOV, Osteoactivin, PD-1, PGRP-5, Serpin A4, sFRP-3, Thrombomodulin, TLR2, TRAIL R1, Transferrin, WIF-1
Quantibody® Human Cytokine Array 7 (40)	ACE-2, Albumin, AMICA, ANG-4, BAFF, CA19-9, CD163, Clusterin, CRTAM, CXCL14, Cystatin C, Decorin, Dkk-3, DLL1, Fetuin A, aFGF, FOLR1, Furin, GASP-1, GASP-2, GCSF R, HAI-2, IL-17B R, IL-27, LAG-3, LDL R, Pepsinogen I, RANK, RBP4, SOST, Syndecan-1, TACI, TFPI, TSP-1, TRAIL R2, TRANCE, Troponin I, uPA, VE-Cadherin, WISP-1
Quantibody® Human Cytokine Array 8 (40)	ANGPTL3, β IG-H3, CA9, Cathepsin B, CD23, CHI3L1, CTLA4, Dkk-4, DPP4, EDA-A2, Epo R, FGF-6, FGF-9, Gas1, IGFBP-5, IL-1F5, IL-1F6, IL-1F7, IL-1F8, IL-1F9, IL-1F10, IL-1R5, IL-17C, IL-18, IL-20, IL-34, IL-5 R α , IL-10 R α , Layilin, Leptin R, Marapsin, Mer, MMP-7, P-Cadherin, Prostatin, PSMA, SIGIRR, TGF β RIII, Tissue Factor, TWEAK

TABLE OF CONTENTS

I.	Overview.....	1
	Introduction.....	3
	How It Works.....	5
II.	Materials Provided.....	6
	Additional Materials Required.....	6
III.	General Considerations.....	7
	A. Preparation of Samples.....	7
	B. Handling Glass Slides.....	7
	C. Incubation.....	7
IV.	Protocol.....	8
	A. Complete Air Dry the Glass Slide.....	8
	B. Prepare Cytokine Standard Dilutions.....	8
	C. Blocking and Incubation.....	9
	D. Incubation with Detection Antibody Cocktail.....	10
	E. Incubation with Cy3 Equivalent Dye-Streptavidin.....	11
	F. Fluorescence Detection.....	11
	G. Data Analysis.....	12
V.	Cytokine Array Map.....	13
VI.	8-Point Standards.....	14
VII.	Quantibody [®] Q-Analyzer.....	20
VIII.	System Recovery.....	20
IX.	Troubleshooting Guide.....	21
X.	Experimental Record Form.....	22

I. 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 array (ELISA). In this method, a target protein is first 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 risky task. To solve this problem take advantage of the innovations in microarray technology over the last decade. 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 in Cell and Nature.

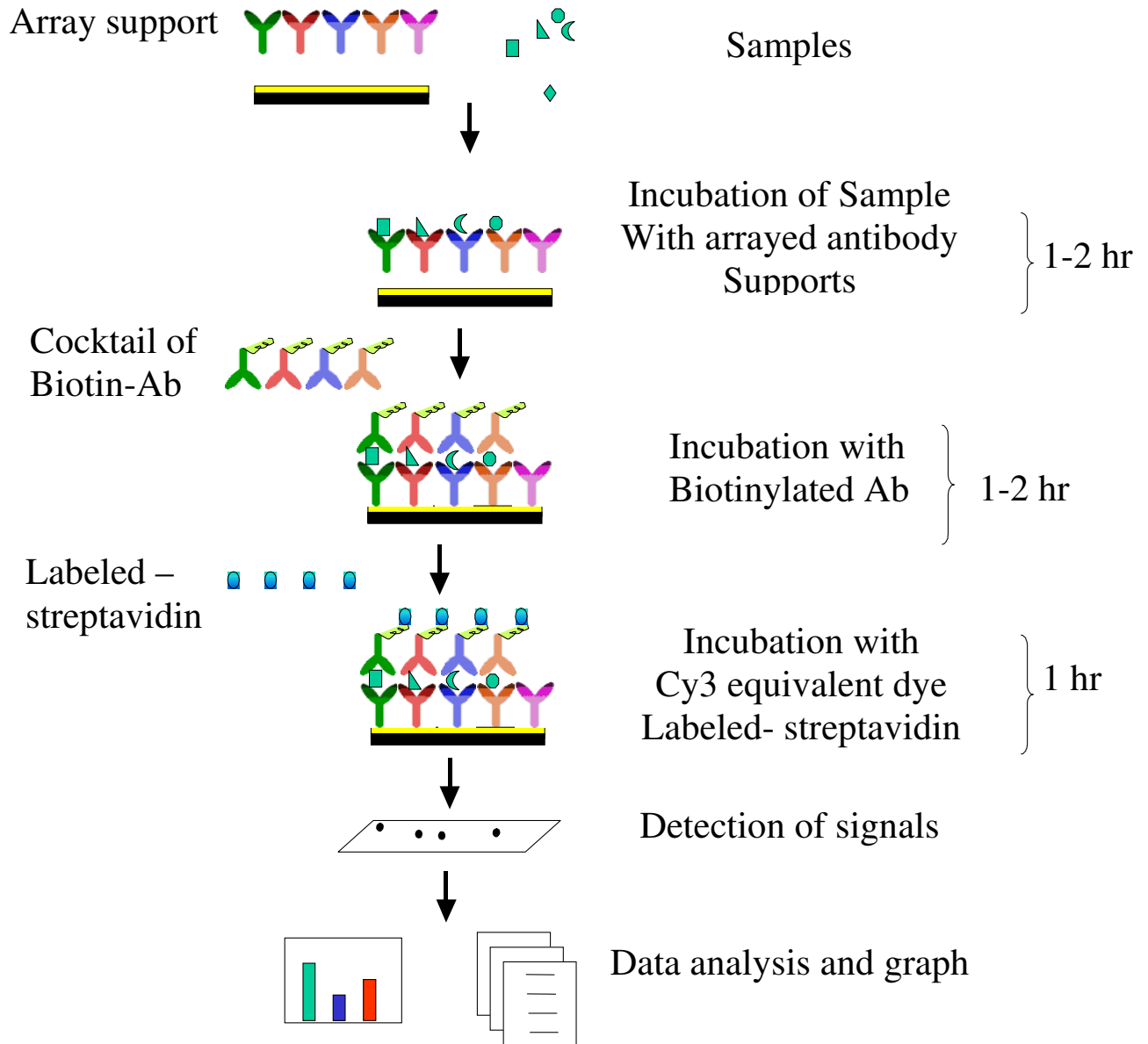
The Quantibody[®] array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and 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 isotope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled Cy3 equivalent dye using a laser scanner. Unlike the traditional ELISA, Quantibody products use 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 400 human, 200 mouse, or 100 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.

How It Works



II. Materials Provided

Upon receipt, all components of the Quantibody® Array kit should be stored at -20°C. At -20°C the kit will retain complete activity for up to 6 months. Once thawed, the glass slide, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at -20°C and all other components may be stored at 4°C. The entire kit should be used within 6 months of purchase.

Components

Item	Description	QAH-CAA-8000-1*	QAH-CAA-8000-2*
1	Quantibody® Array Glass Slide	9	18
2	Sample Diluent	9	9
3	20X Wash Buffer I	18	27
4	20X Wash Buffer II	9	19
5	Lyophilized cytokine standard mix	9	18
6	Detection antibody cocktail	9	18
7	Cy3 equivalent dye-conjugated Streptavidin	9	18
8	Slide Washer/Dryer	9	9
9	Adhesive device sealer	45	90
10	Manual	9	9

*Independent sets of reagents for the different arrays were shipped in different boxes. Among all the reagents, the glass slide, lyophilized cytokine standard mix, and detection antibody cocktail are array specific, while all other reagents are interchangeable between the arrays.

Note: See Section VI for detailed cytokine concentrations after reconstitution.

Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- ddH₂O
- 1.5ml Polypropylene microcentrifuge tubes

III. 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 the readings 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 latex free gloves.
- Handle glass slide in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, this will enable you to distinguish one slide from the other.

C. Incubation

- Completely cover the array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle 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 7 (blocking and incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4⁰C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

IV. Protocol

READ ENTIRE PROTOCOL BEFORE STARTING

*Note: There are nine sets of reagents for the nine **different arrays**. Be careful to use the correct glass slide, lyophilized cytokine standard, and the detection antibody cocktail for the corresponding array. The following is the procedure for processing any one of the arrays in the kit.*

A. Completely air dry the glass slide

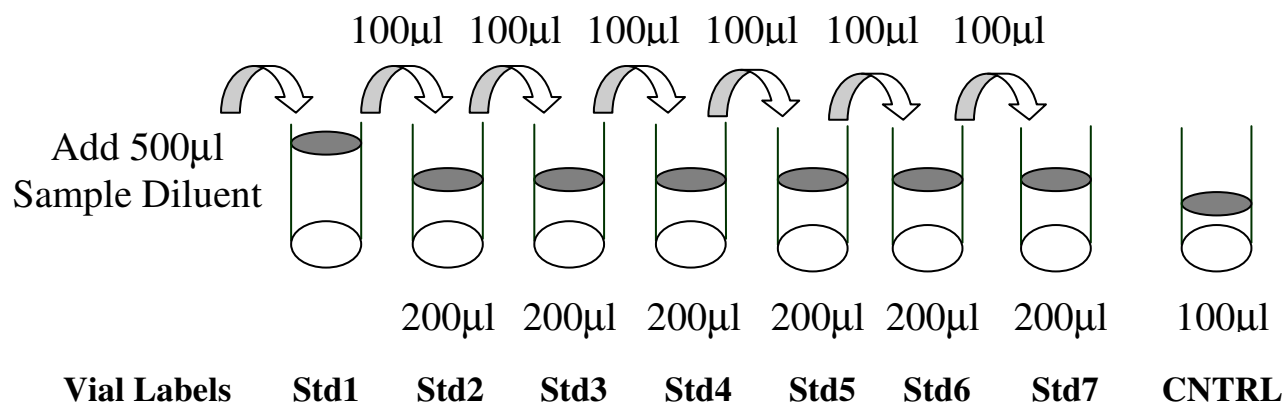
1. Take out the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

Note: Incomplete drying of slides before use may cause the formation of streaks or “comet tails” on a slide.

B. Prepare Cytokine Standard Dilutions

Note: There is only one vial of standard provided in the two-slide kit, this 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 for future use.

Prepare serial dilution of cytokine standards



2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500 μ l 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 μ l Sample Diluent to each of the tubes.
4. Pipette 100 μ l Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100 μ l Std2 to tube Std3 and so on.
5. Add 100 μ l 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.

Note: 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 VI.

C. Blocking and Incubation

6. Add 100 μ l Sample Diluent into each well and incubate at room temperature for 30 min to block slides.
7. Decant buffer from each well. Add 100 μ l standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour. (Be careful to use the corresponding cytokine standard for the matching glass slide.)

Note: We recommend using 50 to 100 μ l of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 μ g/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 μ l of sample or reagent is used.

Note: This step may be done overnight at 4⁰C. The longer incubation time will result in higher overall signals.

8. Wash:

- Calculate the volumes of Wash Buffers required based on the number of samples being processed and the entire remaining protocol described below.
- Dilute 20x Wash Buffer I and 20x Wash Buffer II separately with ddH₂O to generate the required volume of 1x Wash Buffer I and 1x Wash Buffer II. For example 100 µl of 20x Wash Buffer I would be diluted to a final volume of 2,000 µl.
- 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 shaking. Completely remove wash buffer after each wash step.
- *(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 shaking 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 shaking. Completely remove wash buffer in each wash step.

Note: Incomplete removal of the wash buffer in each wash step may cause “dark spots”. (Background signal is higher than that of the spot.)

D. Incubation with detection antibody cocktail and 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. *(Be careful to use the corresponding detection cocktail for the matching glass slide.)*

Note: incubation may be done at 4⁰C for overnight.

11. Decant the samples from each well, and wash 5 times with 150 μ l of 1x Wash Buffer I and then 2 times with 150 μ l of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

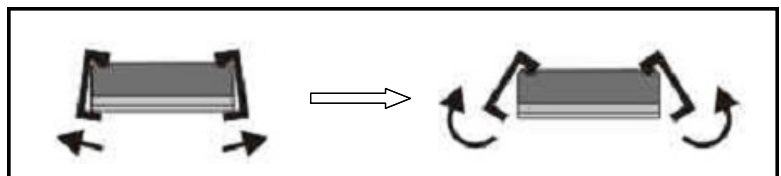
E. Incubation with Cy3 equivalent dye -Streptavidin and wash

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
13. Add 80 μ l 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 with 150 μ l of 1x Wash Buffer I at room temperature with gentle shaking. 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) with gentle shaking at room temperature for 5 minutes then decant Wash Buffer II.

17. Remove buffer droplets from the slide completely by one of the following ways:
- Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
 - Or, dry the glass slide by a compressed N₂ stream.
 - Or gently apply suction with a pipette to remove water droplets. Do not touch the array, only the sides.
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength 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.

Note: If the signal intensity for different cytokines 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 with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). For quantitative data analysis, our Quantibody[®] Q-Analyzer software is available. It gives visual output as well as digital values. More information can be found in section VIII.

V. Array Map

QAH-CYT-8

	1,2,3,4	5,6,7,8	9,10,11,12
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-5 Ra	IL-10 Ra
k	Layilin	Leptin R	Marapsin
l	Mer	MMP-7	P-Cadherin
m	Prostasin	PSMA	SIGIRR
n	TGFb RIII	TF	TWEAK

QAH-CYT-7

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	ACE-2
b	Albumin	AMICA	ANG-4
c	BAFF	CA19-9	CD163
d	Clusterin	CRTAM	CXCL14
e	Cystatin C	Decorin	Dkk-3
f	DLL1	Fetuin A	aFGF
g	FOLR1	Furin	GASP-1
h	GASP-2	G-CSF R	HAI-2
i	IL-17B R	IL-27	LAG-3
j	LDL R	Pepsinogen I	RANK
k	RBP4	SOST	Syndecan-1
l	TACI	TFPI	TSP-1
m	TRAIL R2	TRANCE	Troponin I
n	uPA	VE-Cadherin	WISP-1

QAH-CYT-6

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	2B4
b	ADAM-9	ANG-2	APRIL
c	BMP-2	BMP-9	C5a
d	Cathepsin L	CD200	CD97
e	Chemerin	DcR3	FABP2
f	FAP	FGF-19	Galectin-3
g	HGF R	IFNa/b R2	IGF-II
h	IGF-II R	IL-1 R6	IL-24
i	IL-33	Kallikrein 14	Legumain
j	LOX-1	MBL	Neprilysin
k	Notch-1	NOV	Osteoactivin
l	PD-1	PGRP-5	Serpin A4
m	sFRP-3	Thrombomodulin	TLR2
n	TRAIL R1	Transferrin	WIF-1

QAH-INF-3

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	BLC
b	Eotaxin	Eotaxin-2	G-CSF
c	GM-CSF	I-309	ICAM-1
d	IFN γ	IL-1 α	IL-1 β
e	IL-1ra	IL-2	IL-4
f	IL-5	IL-6	IL-6sR
g	IL-7	IL-8	IL-10
h	IL-11	IL-12p40	IL-12p70
i	IL-13	IL-15	IL-16
j	IL-17	MCP-1	MCSF
k	MIG	MIP-1 α	MIP-1 β
l	MIP-1 δ	PDGF-BB	RANTES
m	TIMP-1	TIMP-2	TNF α
n	TNF β	TNF RI	TNF RII

QAH-GF-1

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	AR
b	BDNF	bFGF	BMP-4
c	BMP-5	BMP-7	b-NGF
d	EGF	EGF R	EG-VEGF
e	FGF-4	FGF-7	GDF-15
f	GDNF	GH	HB-EGF
g	HGF	IGFBP-1	IGFBP-2
h	IGFBP-3	IGFBP-4	IGFBP-6
i	IGF-I	Insulin	MCF R
j	NGF R	NT-3	NT-4
k	OPG	PDGF-AA	PIGF
l	SCF	SCF R	TGF α
m	TGF β	TGF β 3	VEGF
n	VEGF R2	VEGF R3	VEGF-D

QAH-CHE-1

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	6Ckine
b	Axl	BTC	CCL28
c	CTACK	CXCL16	ENA-78
d	Eotaxin-3	GCP-2	GRO
e	HCC-1	HCC-4	IL-9
f	IL-17F	IL-18 BP α	IL-28A
g	IL-29	IL-31	IP-10
h	I-TAC	LIF	LIGHT
i	Lymphotactin	MCP-2	MCP-3
j	MCP-4	MDC	MIF
k	MIP-3 α	MIP-3 β	MPIF-1
l	MSP α	NAP-2	OPN
m	PARC	PF4	SDF-1 α
n	TARC	TECK	TSLP

QAH-REC-1

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	4-1BB
b	ALCAM	B7-1	BCMA
c	CD14	CD30	CD40 L
d	CEACAM-1	DR6	Dik
e	Endoglin	ErbB3	E-Selectin
f	Fas	Fit-3L	GITR
g	HVEM	ICAM-3	IL-1 R4
h	IL-1 RI	IL-2 Ry	IL-10 R β
i	IL-17R	IL-21R	LIMPII
j	Lipocalin-2	L-Selectin	LYVE-1
k	MICA	MICB	NRG1- β 1
l	PDGF R β	PECAM-1	RAGE
m	TIM-1	TRAIL R3	Trappin-2
n	uPAR	VCAM-1	XEDAR

QAH-CYT-4

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	Activin A
b	AgRP	ANG	ANG-1
c	Angiostatin	Cathepsin S	CD 40
d	Cripto-1	DAN	DKK-1
e	E-Cadherin	EpcAM	FAS L
f	Fcr RIIB/C	Follistatin	Galectin-7
g	ICAM-2	IL-13 R1	IL-13 R2
h	IL-17B	IL-2 Ra	IL-2 Rb
i	IL-23	LAP	NrCAM
j	PAI	PDGF-AB	Resistin
k	SDF-1b	spp130	Shh N
l	Siglec-5	ST2	TGF-b2
m	Tie-2	TPO	TRAIL-R4
n	TREM-1	VEGF-C	VEGF-R1

QAH-CYT-5

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	Adiponectin
b	Adipsin	AFP	ANGPTL4
c	B2M	BCAM	CA125
d	CA15-3	CEA	CRP
e	ErbB2	Ferritin	FSH
f	GRO α	HCGb	IGF-I SR
g	IL-1 sRII	IL-3	IL-18 Rb
h	IL-21	Leptin	MMP-1
i	MMP-2	MMP-3	MMP-8
j	MMP-9	MMP-10	MMP-13
k	NCAM-1	Nidogen-1	NSE
l	OSM	Procalcitonin	Prolactin
m	PSA	Siglec-9	TACE
n	Thyroglobulin	TIMP-4	TSH

VI. 8-Point Standards

After reconstitution of the lyophilized cytokine standard mix, the 8-point cytokine concentration used for generating the standard curve of a given antigen are listed below.

QAH-INF-3 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
BLC	0	3	8	25	74	222	667	2,000
Eotaxin	0	5	16	49	148	444	1,333	4,000
Eotaxin-2	0	1	4	12	37	111	333	1,000
G-CSF	0	27	82	247	741	2,222	6,667	20,000
GM-CSF	0	1	4	12	37	111	333	1,000
I-309	0	5	16	49	148	444	1,333	4,000
ICAM-1	0	137	412	1,235	3,704	11,111	33,333	100,000
IFNg	0	3	8	25	74	222	667	2,000
IL-1a	0	3	8	25	74	222	667	2,000
IL-1b	0	1	4	12	37	111	333	1,000
IL-1ra	0	3	8	25	74	222	667	2,000
IL-2	0	3	8	25	74	222	667	2,000
IL-4	0	3	8	25	74	222	667	2,000
IL-5	0	5	16	49	148	444	1,333	4,000
IL-6	0	3	8	25	74	222	667	2,000
IL-6sR	0	14	41	123	370	1,111	3,333	10,000
IL-7	0	5	16	49	148	444	1,333	4,000
IL-8	0	1	2	6	19	56	167	500
IL-10	0	5	16	49	148	444	1,333	4,000
IL-11	0	27	82	247	741	2,222	6,667	20,000
IL-12p40	0	14	41	123	370	1,111	3,333	10,000
IL-12p70	0	1	2	6	19	56	167	500
IL-13	0	1	4	12	37	111	333	1,000
IL-15	0	5	16	49	148	444	1,333	4,000
IL-16	0	7	21	62	185	556	1,667	5,000
IL-17	0	5	16	49	148	444	1,333	4,000
MCP-1	0	3	8	25	74	222	667	2,000
MCSF	0	5	16	49	148	444	1,333	4,000
MIG	0	7	21	62	185	556	1,667	5,000
MIP-1a	0	14	41	123	370	1,111	3,333	10,000
MIP-1b	0	1	4	12	37	111	333	1,000
MIP-1d	0	14	41	123	370	1,111	3,333	10,000
PDGF-BB	0	3	8	25	74	222	667	2,000
RANTES	0	27	82	247	741	2,222	6,667	20,000
TIMP-1	0	55	165	494	1,481	4,444	13,333	40,000
TIMP-2	0	55	165	494	1,481	4,444	13,333	40,000
TNFa	0	3	8	25	74	222	667	2,000
TNFB	0	27	82	247	741	2,222	6,667	20,000
TNF RI	0	55	165	494	1,481	4,444	13,333	40,000
TNF RII	0	55	165	494	1,481	4,444	13,333	40,000

QAH-GF-1 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
AR	0	14	41	123	370	1,111	3,333	10,000
BDNF	0	3	8	25	74	222	667	2,000
bFGF	0	27	82	247	741	2,222	6,667	20,000
BMP-4	0	137	412	1,235	3,704	11,111	33,333	100,000
BMP-5	0	137	412	1,235	3,704	11,111	33,333	100,000
BMP-7	0	55	165	494	1,481	4,444	13,333	40,000
β -NGF	0	14	41	123	370	1,111	3,333	10,000
EGF	0	0	1	2	7	22	67	200
EGF R	0	14	41	123	370	1,111	3,333	10,000
EG-VEGF	0	14	41	123	370	1,111	3,333	10,000
FGF-4	0	137	412	1,235	3,704	11,111	33,333	100,000

FGF-7	0	14	41	123	370	1,111	3,333	10,000
GDF-15	0	3	8	25	74	222	667	2,000
GDNF	0	5	16	49	148	444	1,333	4,000
GH	0	14	41	123	370	1,111	3,333	10,000
HB-EGF	0	14	41	123	370	1,111	3,333	10,000
HGF	0	5	16	49	148	444	1,333	4,000
IGFBP-1	0	7	21	62	185	556	1,667	5,000
IGFBP-2	0	27	82	247	741	2,222	6,667	20,000
IGFBP-3	0	274	823	2,469	7,407	22,222	66,667	200,000
IGFBP-4	0	274	823	2,469	7,407	22,222	66,667	200,000
IGFBP-6	0	137	412	1,235	3,704	11,111	33,333	100,000
IGF-I	0	27	82	247	741	2,222	6,667	20,000
Insulin	0	27	82	247	741	2,222	6,667	20,000
MCF R	0	55	165	494	1,481	4,444	13,333	40,000
NGF R	0	14	41	123	370	1,111	3,333	10,000
NT-3	0	55	165	494	1,481	4,444	13,333	40,000
NT-4	0	14	41	123	370	1,111	3,333	10,000
OPG	0	5	16	49	148	444	1,333	4,000
PDGF-AA	0	14	41	123	370	1,111	3,333	10,000
PIGF	0	5	16	49	148	444	1,333	4,000
SCF	0	14	41	123	370	1,111	3,333	10,000
SCF R	0	27	82	247	741	2,222	6,667	20,000
TGF α	0	14	41	123	370	1,111	3,333	10,000
TGF β 1	0	137	412	1,235	3,704	11,111	33,333	100,000
TGF β 3	0	55	165	494	1,481	4,444	13,333	40,000
VEGF	0	14	41	123	370	1,111	3,333	10,000
VEGF R2	0	14	41	123	370	1,111	3,333	10,000
VEGF R3	0	55	165	494	1,481	4,444	13,333	40,000
VEGF-D	0	27	82	247	741	2,222	6,667	20,000

QAH-CHE-1 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
6Ckine	0	55	165	494	1,481	4,444	13,333	40,000
Axl	0	5	16	49	148	444	1,333	4,000
BTC	0	27	82	247	741	2,222	6,667	20,000
CCL28	0	55	165	494	1,481	4,444	13,333	40,000
CTACK	0	69	206	617	1,852	5,556	16,667	50,000
CXCL16	0	27	82	247	741	2,222	6,667	20,000
ENA-78	0	14	41	123	370	1,111	3,333	10,000
Eotaxin-3	0	27	82	247	741	2,222	6,667	20,000
GCP-2	0	14	41	123	370	1,111	3,333	10,000
GRO	0	1	4	12	37	111	333	1,000
HCC-1	0	5	16	49	148	444	1,333	4,000
HCC-4	0	14	41	123	370	1,111	3,333	10,000
IL-9	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-17F	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-18 BPa	0	82	247	741	2,222	6,667	20,000	60,000
IL-28A	0	14	41	123	370	1,111	3,333	10,000
IL-29	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-31	0	55	165	494	1,481	4,444	13,333	40,000
IP-10	0	14	41	123	370	1,111	3,333	10,000
I-TAC	0	14	41	123	370	1,111	3,333	10,000
LIF	0	18	53	160	481	1,444	4,333	13,000
LIGHT	0	14	41	123	370	1,111	3,333	10,000
Lymphotactin	0	137	412	1,235	3,704	11,111	33,333	100,000
MCP-2	0	3	8	25	74	222	667	2,000
MCP-3	0	5	16	49	148	444	1,333	4,000
MCP-4	0	14	41	123	370	1,111	3,333	10,000
MDC	0	14	41	123	370	1,111	3,333	10,000
MIF	0	5	16	49	148	444	1,333	4,000
MIP-3a	0	5	16	49	148	444	1,333	4,000
MIP-3b	0	27	82	247	741	2,222	6,667	20,000
MPIF-1	0	14	41	123	370	1,111	3,333	10,000
MSPa	0	137	412	1,235	3,704	11,111	33,333	100,000
NAP-2	0	5	16	49	148	444	1,333	4,000
OPN	0	137	412	1,235	3,704	11,111	33,333	100,000
PARC	0	5	16	49	148	444	1,333	4,000
PF4	0	137	412	1,235	3,704	11,111	33,333	100,000
SDF-1a	0	14	41	123	370	1,111	3,333	10,000

TARC	0	14	41	123	370	1,111	3,333	10,000
TECK	0	137	412	1,235	3,704	11,111	33,333	100,000
TSLP	0	14	41	123	370	1,111	3,333	10,000

QAH-REC-1 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
4-1BB	0	14	41	123	370	1,111	3,333	10,000
ALCAM	0	14	41	123	370	1,111	3,333	10,000
B7-1	0	14	41	123	370	1,111	3,333	10,000
BCMA	0	27	82	247	741	2,222	6,667	20,000
CD14	0	14	41	123	370	1,111	3,333	10,000
CD30	0	14	41	123	370	1,111	3,333	10,000
CD40 L	0	14	41	123	370	1,111	3,333	10,000
CEACAM-1	0	14	41	123	370	1,111	3,333	10,000
DR6	0	5	16	49	148	444	1,333	4,000
Dtk	0	27	82	247	741	2,222	6,667	20,000
Endoglin	0	5	16	49	148	444	1,333	4,000
ErbB3	0	27	82	247	741	2,222	6,667	20,000
E-Selectin	0	55	165	494	1,481	4,444	13,333	40,000
Fas	0	3	8	25	74	222	667	2,000
Fit-3L	0	3	8	25	74	222	667	2,000
GITR	0	14	41	123	370	1,111	3,333	10,000
HVEM	0	55	165	494	1,481	4,444	13,333	40,000
ICAM-3	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-1 R4	0	5	16	49	148	444	1,333	4,000
IL-1 RI	0	5	16	49	148	444	1,333	4,000
IL-2 R γ	0	14	41	123	370	1,111	3,333	10,000
IL-10 R β	0	5	16	49	148	444	1,333	4,000
IL-17R	0	14	41	123	370	1,111	3,333	10,000
IL-21R	0	27	82	247	741	2,222	6,667	20,000
LIMPII	0	5	16	49	148	444	1,333	4,000
Lipocalin-2	0	1	4	12	37	111	333	1,000
L-Selectin	0	137	412	1,235	3,704	11,111	33,333	100,000
LYVE-1	0	3	8	25	74	222	667	2,000
MICA	0	14	41	123	370	1,111	3,333	10,000
MICB	0	21	62	185	556	1,667	5,000	15,000
NRG1- β 1	0	21	62	185	556	1,667	5,000	15,000
PDGF R β	0	137	412	1,235	3,704	11,111	33,333	100,000
PECAM-1	0	27	82	247	741	2,222	6,667	20,000
RAGE	0	14	41	123	370	1,111	3,333	10,000
TIM-1	0	14	41	123	370	1,111	3,333	10,000
TRAIL R3	0	7	21	62	185	556	1,667	5,000
Trappin-2	0	14	41	123	370	1,111	3,333	10,000
uPAR	0	55	165	494	1,481	4,444	13,333	40,000
VCAM-1	0	274	823	2,469	7,407	22,222	66,667	200,000
XEDAR	0	14	41	123	370	1,111	3,333	10,000

QAH-CYT-4 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
Activin A	0	137	412	1,235	3,704	11,111	33,333	100,000
AgRP	0	14	41	123	370	1,111	3,333	10,000
ANG	0	3	8	25	74	222	667	2,000
ANG-1	0	55	165	494	1,481	4,444	13,333	40,000
Angiostatin	0	1,372	4,115	12,346	37,037	111,111	333,333	1,000,000
Cathepsin S	0	14	41	123	370	1,111	3,333	10,000
CD 40	0	14	41	123	370	1,111	3,333	10,000
Cripto-1	0	14	41	123	370	1,111	3,333	10,000
DAN	0	55	165	494	1,481	4,444	13,333	40,000
DKK-1	0	110	329	988	2,963	8,889	26,667	80,000
E-Cadherin	0	110	329	988	2,963	8,889	26,667	80,000
EpCAM	0	27	82	247	741	2,222	6,667	20,000
FAS L	0	3	8	25	74	222	667	2,000
Fcr RIIB/C	0	14	41	123	370	1,111	3,333	10,000
Follistatin	0	55	165	494	1,481	4,444	13,333	40,000
Galectin-7	0	137	412	1,235	3,704	11,111	33,333	100,000
ICAM-2	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-13 R1	0	14	41	123	370	1,111	3,333	10,000
IL-13 R2	0	27	82	247	741	2,222	6,667	20,000

IL-17B	0	55	165	494	1,481	4,444	13,333	40,000
IL-2 Ra	0	14	41	123	370	1,111	3,333	10,000
IL-2 Rb	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-23	0	55	165	494	1,481	4,444	13,333	40,000
LAP	0	5	16	49	148	444	1,333	4,000
NrCAM	0	27	82	247	741	2,222	6,667	20,000
PAI-I	0	55	165	494	1,481	4,444	13,333	40,000
PDGF-AB	0	14	41	123	370	1,111	3,333	10,000
Resistin	0	27	82	247	741	2,222	6,667	20,000
SDF-1b	0	55	165	494	1,481	4,444	13,333	40,000
sgp130	0	110	329	988	2,963	8,889	26,667	80,000
Shh N	0	55	165	494	1,481	4,444	13,333	40,000
Siglec-5	0	14	41	123	370	1,111	3,333	10,000
ST2	0	5	16	49	148	444	1,333	4,000
TGF-b2	0	55	165	494	1,481	4,444	13,333	40,000
Tie-2	0	14	41	123	370	1,111	3,333	10,000
TPO	0	274	823	2,469	7,407	22,222	66,667	200,000
TRAIL-R4	0	11	33	99	296	889	2,667	8,000
TREM-1	0	27	82	247	741	2,222	6,667	20,000
VEGF-C	0	27	82	247	741	2,222	6,667	20,000
VEGF-R1	0	55	165	494	1,481	4,444	13,333	40,000

QAH-CYT-5 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
Adiponectin	0	137	412	1,235	3,704	11,111	33,333	100,000
Adipsin	0	27	82	247	741	2,222	6,667	20,000
AFP	0	14	41	123	370	1,111	3,333	10,000
ANGPTL4	0	549	1,646	4,938	14,815	44,444	133,333	400,000
B2M	0	14	41	123	370	1,111	3,333	10,000
BCAM	0	55	165	494	1,481	4,444	13,333	40,000
CA125	0	137	412	1,235	3,704	11,111	33,333	100,000
CA15-3	0	41	123	370	1,111	3,333	10,000	30,000
CEA	0	27	82	247	741	2,222	6,667	20,000
CRP	0	14	41	123	370	1,111	3,333	10,000
ErbB2	0	14	41	123	370	1,111	3,333	10,000
Ferritin	0	1,097	3,292	9,877	29,630	88,889	266,667	800,000
FSH	0	14	41	123	370	1,111	3,333	10,000
GRO α	0	137	412	1,235	3,704	11,111	33,333	100,000
HCG β	0	27	82	247	741	2,222	6,667	20,000
IGF-I SR	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-1 sRII	0	27	82	247	741	2,222	6,667	20,000
IL-3	0	14	41	123	370	1,111	3,333	10,000
IL-18 R β	0	27	82	247	741	2,222	6,667	20,000
IL-21	0	137	412	1,235	3,704	11,111	33,333	100,000
Leptin	0	55	165	494	1,481	4,444	13,333	40,000
MMP-1	0	55	165	494	1,481	4,444	13,333	40,000
MMP-2	0	137	412	1,235	3,704	11,111	33,333	100,000
MMP-3	0	55	165	494	1,481	4,444	13,333	40,000
MMP-8	0	14	41	123	370	1,111	3,333	10,000
MMP-9	0	27	82	247	741	2,222	6,667	20,000
MMP-10	0	14	41	123	370	1,111	3,333	10,000
MMP-13	0	14	41	123	370	1,111	3,333	10,000
NCAM-1	0	274	823	2,469	7,407	22,222	66,667	200,000
Nidogen-1	0	27	82	247	741	2,222	6,667	20,000
NSE	0	137	412	1,235	3,704	11,111	33,333	100,000
OSM	0	14	41	123	370	1,111	3,333	10,000
Procalcitonin	0	137	412	1,235	3,704	11,111	33,333	100,000
Prolactin	0	549	1,646	4,938	14,815	44,444	133,333	400,000
PSA	0	27	82	247	741	2,222	6,667	20,000
Siglec-9	0	55	165	494	1,481	4,444	13,333	40,000
TACE	0	137	412	1,235	3,704	11,111	33,333	100,000
Thyroglobulin	0	137	412	1,235	3,704	11,111	33,333	100,000
TIMP-4	0	27	82	247	741	2,222	6,667	20,000
TSH	0	27	82	247	741	2,222	6,667	20,000

QAH-CYT-6 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
2B4	0	14	41	123	370	1,111	3,333	10,000

ADAM-9	0	137	412	1,235	3,704	11,111	33,333	100,000
ANG-2	0	27	82	247	741	2,222	6,667	20,000
APRIL	0	274	823	2,469	7,407	22,222	66,667	200,000
BMP-2	0	137	412	1,235	3,704	11,111	33,333	100,000
BMP-9	0	5	16	49	148	444	1,333	4,000
C5a	0	14	41	123	370	1,111	3,333	10,000
Cathepsin L	0	14	41	123	370	1,111	3,333	10,000
CD200	0	137	412	1,235	3,704	11,111	33,333	100,000
CD97	0	137	412	1,235	3,704	11,111	33,333	100,000
Chemerin	0	274	823	2,469	7,407	22,222	66,667	200,000
DcR3	0	274	823	2,469	7,407	22,222	66,667	200,000
FABP2	0	137	412	1,235	3,704	11,111	33,333	100,000
FAP	0	27	82	247	741	2,222	6,667	20,000
FGF-19	0	27	82	247	741	2,222	6,667	20,000
Galectin-3	0	5	16	49	148	444	1,333	4,000
HGF R	0	5	16	49	148	444	1,333	4,000
IFN α / β R2	0	137	412	1,235	3,704	11,111	33,333	100,000
IGF-II	0	137	412	1,235	3,704	11,111	33,333	100,000
IGF-II R	0	27	82	247	741	2,222	6,667	20,000
IL-1 R6	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-24	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-33	0	14	41	123	370	1,111	3,333	10,000
Kallikrein 14	0	5	16	49	148	444	1,333	4,000
Legumain	0	14	41	123	370	1,111	3,333	10,000
LOX-1	0	3	8	25	74	222	667	2,000
MBL	0	1	4	12	37	111	333	1,000
Neprilysin	0	27	82	247	741	2,222	6,667	20,000
Notch-1	0	5	16	49	148	444	1,333	4,000
NOV	0	5	16	49	148	444	1,333	4,000
Osteoactivin	0	14	41	123	370	1,111	3,333	10,000
PD-1	0	5	16	49	148	444	1,333	4,000
PGRP-5	0	1	4	12	37	111	333	1,000
Serpin A4	0	14	41	123	370	1,111	3,333	10,000
sFRP-3	0	137	412	1,235	3,704	11,111	33,333	100,000
Thrombomodulin	0	137	412	1,235	3,704	11,111	33,333	100,000
TLR2	0	27	82	247	741	2,222	6,667	20,000
TRAIL R1	0	14	41	123	370	1,111	3,333	10,000
Transferrin	0	137	412	1,235	3,704	11,111	33,333	100,000
WIF-1	0	27	82	247	741	2,222	6,667	20,000

QAH-CYT-7 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
ACE-2	0	549	1,646	4,938	14,815	44,444	133,333	400,000
Albumin	0	27	82	247	741	2,222	6,667	20,000
AMICA	0	27	82	247	741	2,222	6,667	20,000
ANG-4	0	27	82	247	741	2,222	6,667	20,000
BAFF	0	14	41	123	370	1,111	3,333	10,000
CA19-9	0	137	412	1,235	3,704	11,111	33,333	100,000
CD163	0	274	823	2,469	7,407	22,222	66,667	200,000
Clusterin	0	14	41	123	370	1,111	3,333	10,000
CRTAM	0	5	16	49	148	444	1,333	4,000
CXCL14	0	137	412	1,235	3,704	11,111	33,333	100,000
Cystatin C	0	137	412	1,235	3,704	11,111	33,333	100,000
Decorin	0	3	8	25	74	222	667	2,000
Dkk-3	0	137	412	1,235	3,704	11,111	33,333	100,000
DLL1	0	27	82	247	741	2,222	6,667	20,000
Fetuin A	0	55	165	494	1,481	4,444	13,333	40,000
aFGF	0	274	823	2,469	7,407	22,222	66,667	200,000
FOLR1	0	137	412	1,235	3,704	11,111	33,333	100,000
Furin	0	274	823	2,469	7,407	22,222	66,667	200,000
GASP-1	0	3	8	25	74	222	667	2,000
GASP-2	0	137	412	1,235	3,704	11,111	33,333	100,000
GCSF R	0	14	41	123	370	1,111	3,333	10,000
HAI-2	0	55	165	494	1,481	4,444	13,333	40,000
IL-17B R	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-27	0	14	41	123	370	1,111	3,333	10,000
LAG-3	0	137	412	1,235	3,704	11,111	33,333	100,000
LDL R	0	3	8	25	74	222	667	2,000
Pepsinogen I	0	137	412	1,235	3,704	11,111	33,333	100,000

RANK	0	137	412	1,235	3,704	11,111	33,333	100,000
RBP4	0	27	82	247	741	2,222	6,667	20,000
SOST	0	55	165	494	1,481	4,444	13,333	40,000
Syndecan-1	0	137	412	1,235	3,704	11,111	33,333	100,000
TACI	0	55	165	494	1,481	4,444	13,333	40,000
TFPI	0	137	412	1,235	3,704	11,111	33,333	100,000
TSP-1	0	137	412	1,235	3,704	11,111	33,333	100,000
TRAIL R2	0	5	16	49	148	444	1,333	4,000
TRANCE	0	55	165	494	1,481	4,444	13,333	40,000
Troponin I	0	274	823	2,469	7,407	22,222	66,667	200,000
uPA	0	5	16	49	148	444	1,333	4,000
VE-Cadherin	0	274	823	2,469	7,407	22,222	66,667	200,000
WISP-1	0	274	823	2,469	7,407	22,222	66,667	200,000

QAH-CYT-8 Serial standard concentration (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
ANGPTL3	0	14	41	123	370	1,111	3,333	10,000
β IG-H3	0	14	41	123	370	1,111	3,333	10,000
CA9	0	14	41	123	370	1,111	3,333	10,000
Cathepsin B	0	14	41	123	370	1,111	3,333	10,000
CD23	0	14	41	123	370	1,111	3,333	10,000
CHI3L1	0	14	41	123	370	1,111	3,333	10,000
CTLA4	0	5	16	49	148	444	1,333	4,000
Dkk-4	0	137	412	1,235	3,704	11,111	33,333	100,000
DPPIV	0	274	823	2,469	7,407	22,222	66,667	200,000
EDA-A2	0	14	41	123	370	1,111	3,333	10,000
Epo R	0	55	165	494	1,481	4,444	13,333	40,000
FGF-6	0	14	41	123	370	1,111	3,333	10,000
FGF-9	0	5	16	49	148	444	1,333	4,000
Gas1	0	137	412	1,235	3,704	11,111	33,333	100,000
IGFBP-5	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-1F5	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-1F6	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-1F7	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-1F8	0	5	16	49	148	444	1,333	4,000
IL-1F9	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-1F10	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-1R5	0	1	4	12	37	111	333	1,000
IL-17C	0	549	1,646	4,938	14,815	44,444	133,333	400,000
IL-18	0	55	165	494	1,481	4,444	13,333	40,000
IL-20	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-34	0	55	165	494	1,481	4,444	13,333	40,000
IL-5 R α	0	549	1,646	4,938	14,815	44,444	133,333	400,000
IL-10 R α	0	274	823	2,469	7,407	22,222	66,667	200,000
Layilin	0	14	41	123	370	1,111	3,333	10,000
Leptin R	0	137	412	1,235	3,704	11,111	33,333	100,000
Marapsin	0	27	82	247	741	2,222	6,667	20,000
Mer	0	14	41	123	370	1,111	3,333	10,000
MMP-7	0	137	412	1,235	3,704	11,111	33,333	100,000
P-Cadherin	0	137	412	1,235	3,704	11,111	33,333	100,000
Prostasin	0	27	82	247	741	2,222	6,667	20,000
PSMA	0	137	412	1,235	3,704	11,111	33,333	100,000
SIGIRR	0	137	412	1,235	3,704	11,111	33,333	100,000
TGF β RIII	0	27	82	247	741	2,222	6,667	20,000
TF	0	5	16	49	148	444	1,333	4,000
TWEAK	0	137	412	1,235	3,704	11,111	33,333	100,000

VII. Quantibody® Q-Analyzer

Quantibody Q-Analyzer is an array specific, Excel-based program. However, it is not a simple calculation macro as it contains sophisticated data analysis.

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.

VIII. System Recovery

The antibody pairs used in the kits have been tested to recognize their specific antigen. The spiking recovery rate of the cytokines by the kits in serum and cell culture media can be found in their individual manuals.

IX. 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	Ensure sufficient incubation time or change sample incubation to an overnight step
	Too low protein concentration in sample	Dilute starting sample less or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between 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 at 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 a new cytokine standard vial for a new experiment. Discard any leftovers
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from drying out during experiment

X. 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		

1	2
3	4
5	6
7	8

Note:

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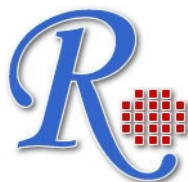
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