Quantibody® Human Cytokine Antibody Array 2000

--Quantitative measurement of 120 Human cytokines

Patent Pending Technology

User Manual (Version July 2010)

Quantibody® Human Cytokine Antibody Array 2000

(Combination of Quantibody® human Inflammation Array 3, human Growth Factor Array 1, and human Chemokine Array 1 to quantitatively measure the concentration of 120 human cytokines)

Cat # QAH-CAA-2000

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)

RayBiotech, Inc.

We Provide You With Excellent Protein Array Systems and Service

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Cytokine Detected	120
Arrays Included	Quantibody® Human Inflammation Array 3, Quantibody® Human Growth Factor Array 1, and Quantibody® Human Chemokine Array 1
Quantibody® Human Inflammation Array 3 (40)	BLC, Eotaxin, Eotaxin-2, G-CSF, GM-CSF, I-309, ICAM-1, IFNγ, IL-1α, IL-1β, 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-1α, MIP-1β, MIP-1δ, PDGF-BB, RANTES, TIMP-1, TIMP-2, TNFα, TNFβ, 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, TGFα, TGFβ1, TGFβ3, 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 BPa, IL-28A, IL-29, IL-31, IP-10, I-TAC, LIF, LIGHT, Lymphotactin, MCP-2, MCP-3, MCP-4, MDC, MIF, MIP-3α, MIP-3β, MPIF-1, MSPα, NAP-2, OPN, PARC, PF4, SDF-1α, TARC, TECK, TSLP
Format	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
Detection Method	Fluorescence with laser scanner: Cy3 equivalent dye
Sample Volume	50 – 100 µl per array
Reproducibility	CV <20%
Assay duration	6 hrs

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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, 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 the traditional method works well for a single protein, the overall procedure is time consuming and requires a lot of sample. With little sample to work with, conservation of precious small quantities becomes a risky task. Take the advantage of advancement in microarray technology over the last decade; more and more choices are available to the scientist today. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which has now been widely applied in the research community with hundreds of peer reviewed publications such as in Cell and Nature.

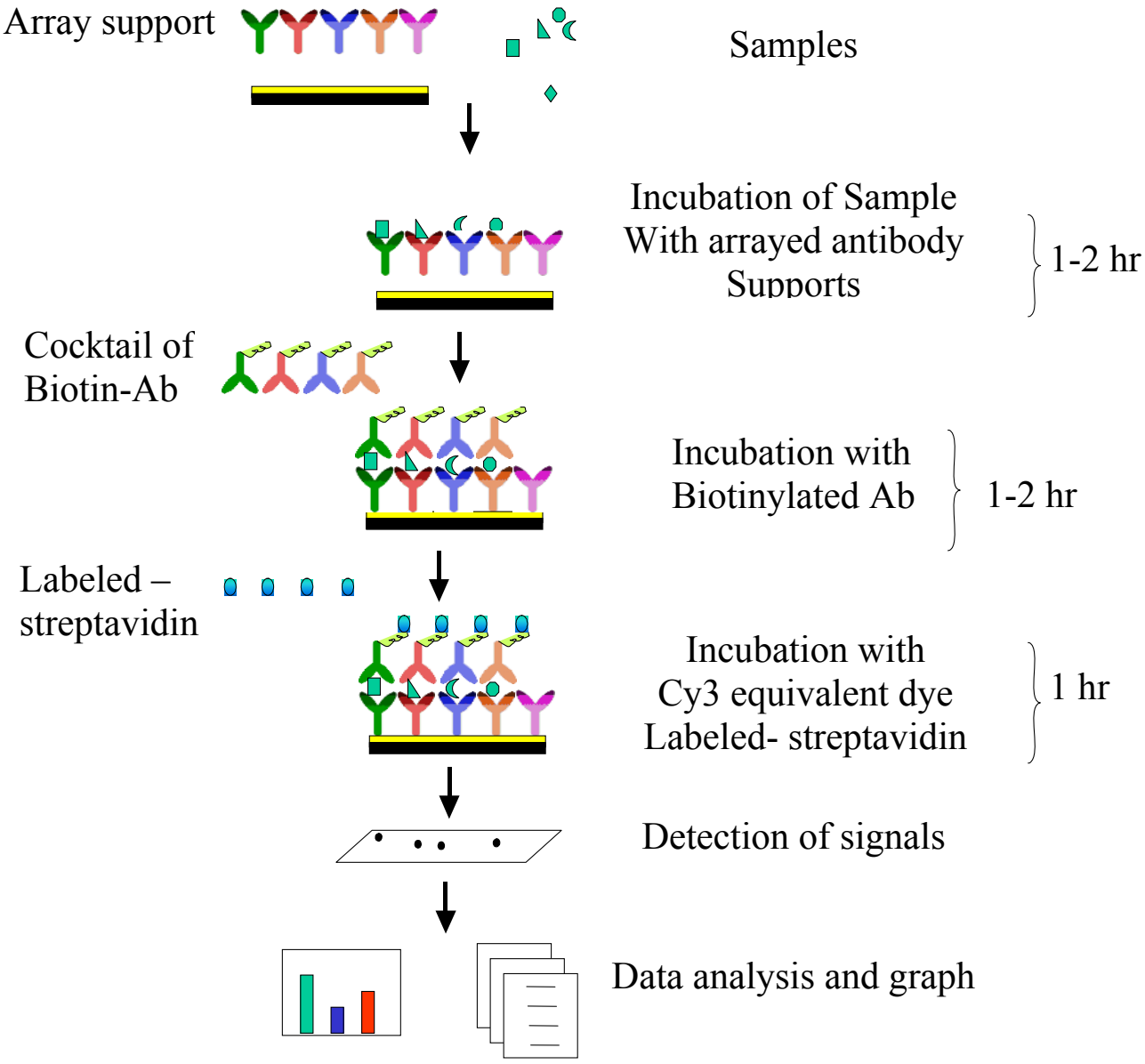
Quantibody® array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity / specificity of ELISA and the high throughput of the 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, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is spotted with 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 in one slide. Four slide chips can be nested into a tray, which matches a standard microplate 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 160 human or 120 mouse 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


Array support
Samples
Incubation of Sample
With arrayed antibody
Supports
1-2 hr
Cocktail of
Biotin-Ab
Incubation with
Biotinylated Ab
1-2 hr
Labeled –
streptavidin
Incubation with
Cy3 equivalent dye
Labeled- streptavidin
1 hr
Detection of signals
Data analysis and graph

II. Materials Provided

Upon receipt, all components of the Quantibody® Array kit should be stored at -20^{0}C. At -20^{0}C the kit will retain complete activity for up to 6 months. Once thawed, the glass chip, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at –20^{0}C and all other components may be stored at 4^{0}C. The entire kit should be used within 6 months of purchase.

Components

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

* There are three independent sets of reagents for Quantibody® human Inflammation Array 3, Quantibody® Human Growth Factor Array 1, and Quantibody® Human Chemokine Array 1. Among all the reagents, the glass chip, lyophilized cytokine standard mix, and detection antibody cocktail are array specific, while all the other reagents are suitable for all the three arrays.

Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- 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 chips

- 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 chip 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, you might not have enough info to distinguish one slide from the other.

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 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 4^0C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

IV. Protocol

Note: There are three sets of reagents for ***three different arrays.*** *Be careful to use the glass chip, lyophilized cytokine standard, and the detection antibody cocktail for the same array. Following is the procedure for processing any one of the arrays in the kit.*

A. Completely air dry the glass chip

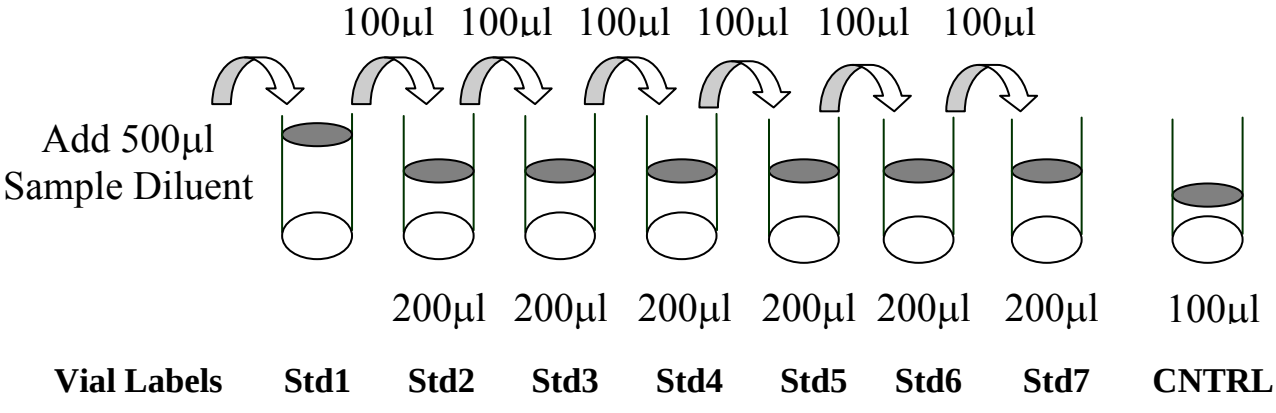
1. Take out the glass chip from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

Note: Incomplete drying of slides before use may cause the formation of "comet tails".

B. Prepare Cytokine Standard Dilutions

Note: Reconstitute the lyophilized standard within one hour of usage.

Prepare serial dilution of cytokine standards



2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500µl 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 200µl Sample Diluent to each of the tubes.

4. Pipette 100µl Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100ul Std2 to tube Std3 and so on.

5. Add 100µl 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 100µl Sample Diluent into each well and incubate at room temperature for 30 min to block slides.

7. Decant buffer from each well. Add 100µl 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 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 if less than 70 ul of sample or reagent is used.

   Note: This step may be done overnight at 4^0C for best results. Longer incubation time is preferable for higher signal.

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

$_2$O.

- (*Optional for Cell and Tissue Lysates*) Put the glass chip 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. Dilute 20x Wash Buffer II with H_2O.

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 4^0C for overnight.

11. Decant the samples from each well, and wash 5 times 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 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.

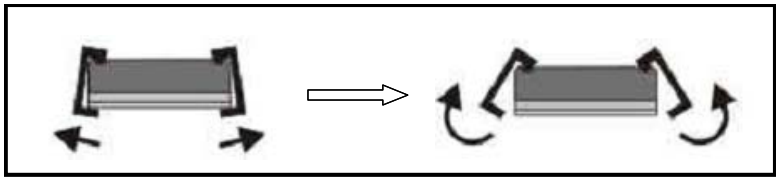
13. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.

14. Decant the samples from each well, and wash 5 times with 150 µl 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, and gently shake at room temperature for 5 minutes.

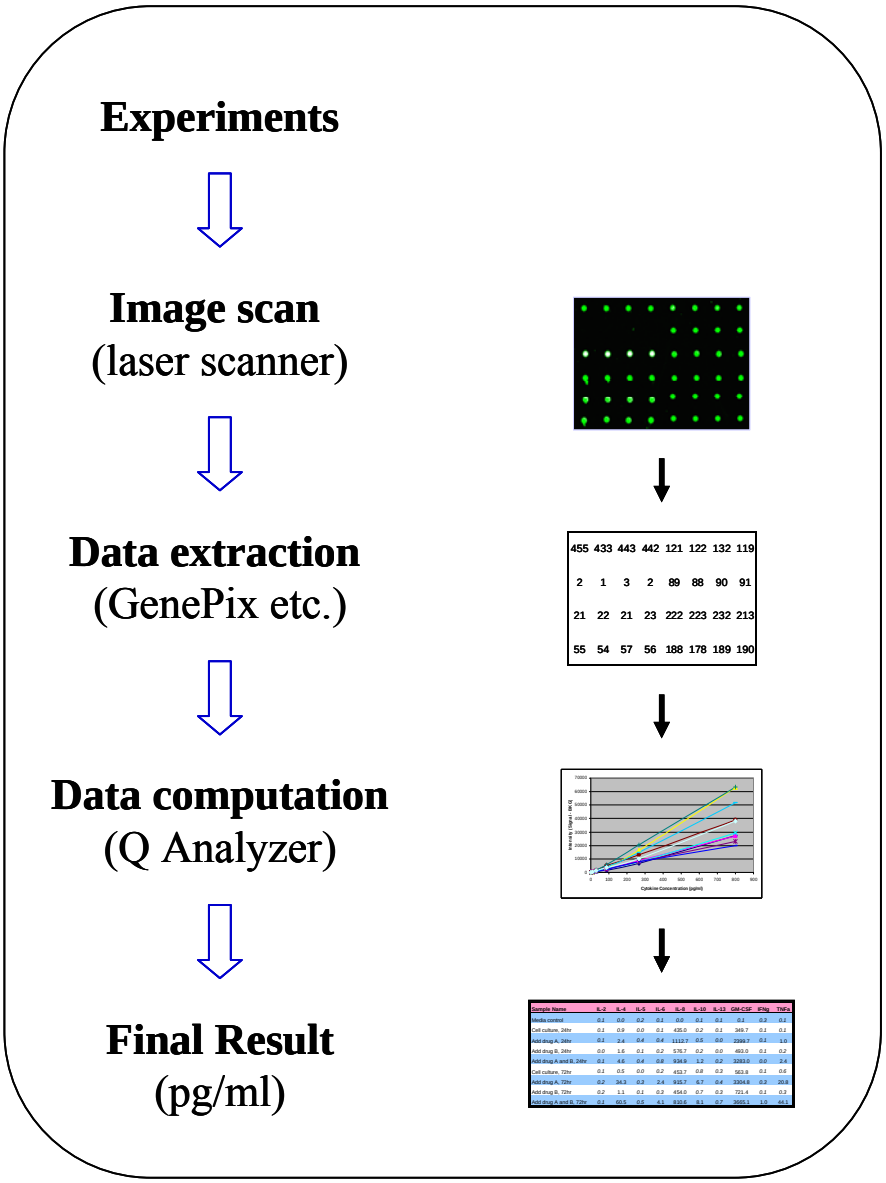
17. Remove water droplets completely by one of the following ways:
    - Put the glass chip into the Slide Washer/Dryer, and dry the glass chip by centrifuge at 1,000 rpm for 3 minutes without cap.
    - Or, dry the glass chip by a compressed N_2 stream.
    - Or gently apply suction with a pipette to 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: 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 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. Cytokine Array Map

QAH-INF-3

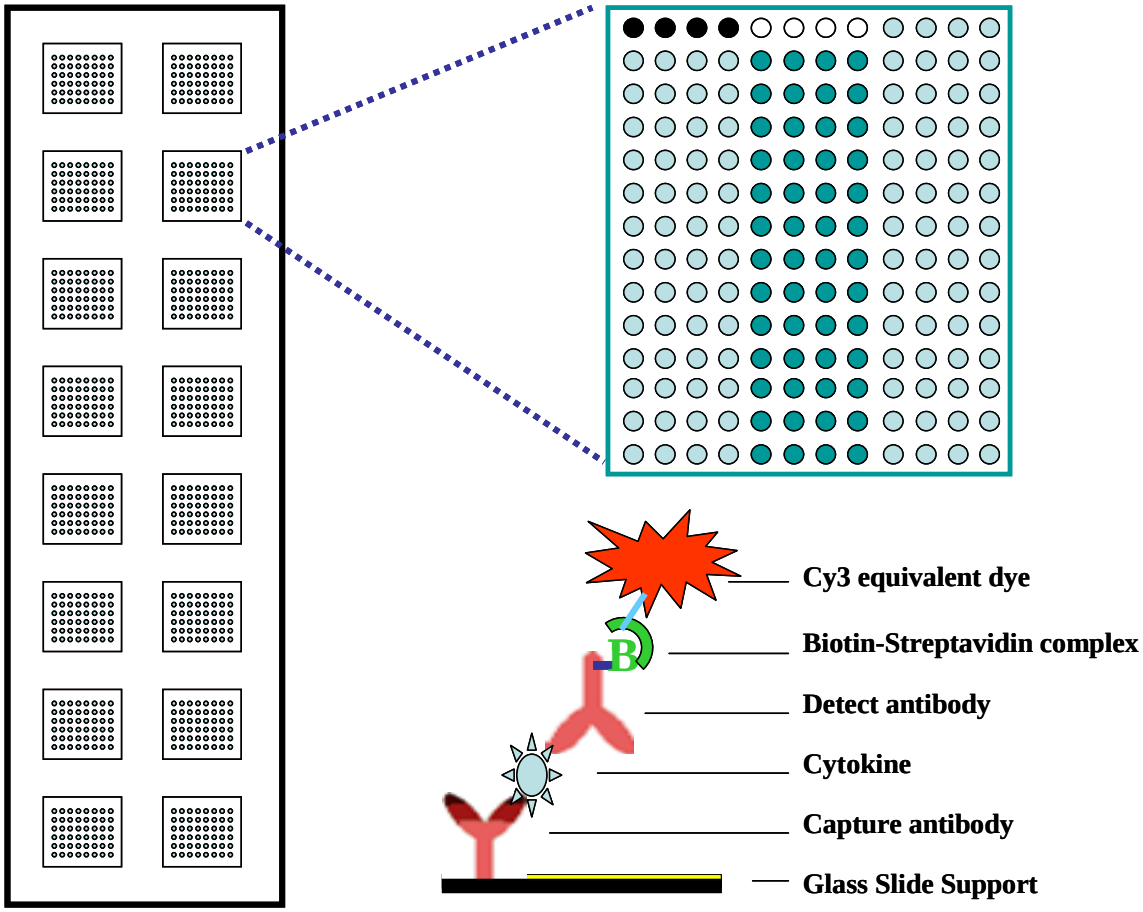
	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	BLC
b	Eotaxin	Eotaxin-2	G-CSF
c	GM-CSF	I-309	ICAM-1
d	IFNg	IL-1a	IL-1b
e	IL-1ra	IL-2	IL-4
f	IL-5	IL-6	IL-6sR
g	IL-7	IL-8	IL-10
h	IL-11	IL-12p40	IL-12p70
i	IL-13	IL-15	IL-16
j	IL-17	MCP-1	MCSF
k	MIG	MIP-1a	MIP-1b
l	MIP-1d	PDGF-BB	RANTES
m	TIMP-1	TIMP-2	TNFa
n	TNFb	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	TGFa
m	TGFb	TGFb3	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 BPa	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-3a	MIP-3b	MPIF-1
l	MSPa	NAP-2	OPN
m	PARC	PF4	SDF-1a
n	TARC	TECK	TSLP



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 is listed below. The detection sensitivity of each protein in one experiment is user dependent. Try our array specific Quantibody Q-Analyzer to see your Limit of Detection (LOD). (Section VIII).

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
TGFα	0	14	41	123	370	1,111	3,333	10,000
TGFβ1	0	137	412	1,235	3,704	11,111	33,333	100,000
TGFβ3	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

VII. 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.

VIII. 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 number of samples.
- *Two Positive Controls*: The program takes 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 those 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.

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 and change sample incubation step to overnight
	Too low protein concentration in sample	Don't make too low dilution or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed covered by reagent	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
	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 that 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 laser power
	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.

X. Select Quantibody Publications

1. Stechova, et al. Influence of Maternal Hyperglycaemia on Cord Blood Mononuclear Cells in Response to Diabetes-associated Autoantigens. *Scandinavian Journal of Immunology*. 2009. 70(2):149-158
2. Willingham, SB et al. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol.* 2009; 183(3):2008-15
3. El Karim et al. Neuropeptides Regulate Expression of Angiogenic Growth Factors in Human Dental Pulp Fibroblasts. *Journal of Endodontics*, 2009; 35(6): 829-833
4. Souquière S. et al. T-Cell tropism of simian T-cell leukaemia virus type 1 and cytokine profiles in relation to proviral load and immunological changes during chronic infection of naturally infected mandrills (*Mandrillus sphinx*). *J Med Primatol*. 2009; 38(4):279-89
5. Sharma, et al. Induction of multiple pro-inflammatory cytokines by respiratory viruses and reversal by standardized *Echinacea*, a potent antiviral herbal extract. *Antiviral Research*. 2009; 83(2)165-170.
6. Altamirano-Dimas, et al. *Echinacea* and anti-inflammatory cytokine responses: Results of a gene and protein array analysis. *Pharmacuetical Biology*. 2009; 47(6): 500-508.
7. Cheung, et al. Cordysinocan, a polysaccharide isolated from cultured *Cordyceps*, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. *Journal of Ethonopharmacology*. 2009; 124(1): 61-68.
8. Du, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. *Alzheimer's and Dementia*. 2009; 4(4): T484-T484.
9. Van Rossum et al. Granulocytosis and thrombocytosis in renal cell carcinoma: a pro-inflammatory cytokine response originating in the tumour. *Neth J Med*. 2009; 67(5):191-4.
10. Zhai, et al. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. *Molecular and Cellular Biology*. 2008; 28(24): 7414-7426.
11. Gao, et al. A Chinese herbal decoction, Danggui Buxue Tang, activates extracellular signal-regulated kinase in cultured T-lymphocytes. *FEBS Letters*, 2007; 581(26): 5087-5093. (This reference validates mulitplex ELISA results for several analytes with standard ELISA test results).
12. Piganelli, et al: Autoreactive T-cell responses: new technology in pursuit of an old nemesis. (Editorial Review) *Pediatric Diabetes* 2007: 8: 249–251

XI. Experiment Record Form

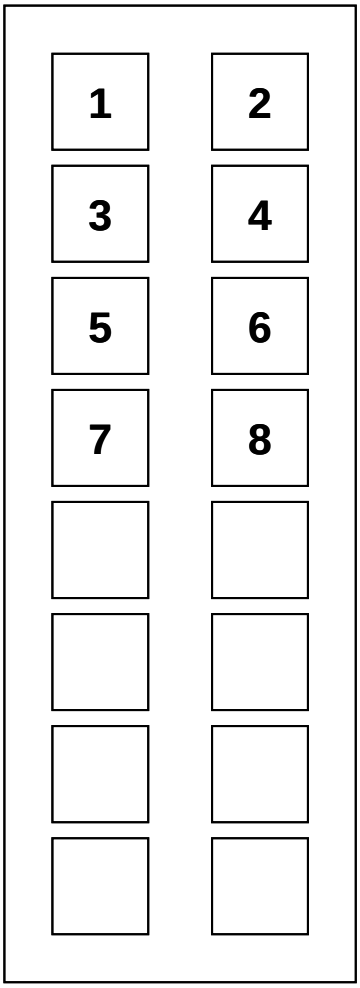
Date: _____

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		



Note:

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