

Quantibody® Human Angiogenesis Array 1000

--- Quantitative measurement of 60 human angiogenesis factors

Patent Pending Technology

User Manual (Version Jul 08)

Quantibody® Human Angiogenesis Array 1000

(Combination of Quantibody® human angiogenesis arrays 2 & 3
to quantitatively measure the concentration of 60 human cytokines)

Cat # QAH-ANG-1000

Quantibody® Human Angiogenesis Array 2 (Cat# QAH-ANG-2)

Quantibody® Human Angiogenesis Array 3 (Cat# QAH-ANG-3)



RayBiotech, Inc.

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

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OVERVIEW

Quantibody® Human Angiogenesis Array 1000

Cytokine Detected	60
Arrays Included	Quantibody® Human Cytokine Arrays 2, and 3
Quantibody® Human Angiogenesis Array 2 (30)	Activin A, AgRP, Angiogenin, ANG-2, ANGPTL4, bFGF, ENA-78, GRO, HB-EGF, HGF, IFN γ , IGF-I, IL-1 α , IL-2, IL-6, IL-8, IL-17, IP-10, Leptin, LIF, MCP-1, PDGF-BB, PIGF, RANTES, TGF β 1, TIMP-1, TIMP-2, TNF α , TNF β , TPO
Quantibody® Human Angiogenesis Array 3 (30)	ANG-1, Angiostatin, CXCL16, EGF, FGF-4, Follistatin, G-CSF, GM-CSF, I-309, IL-1 β , IL-4, IL-10, IL-12p40, IL-12p70, I-TAC, MCP-2, MCP-3, MCP-4, MMP-1, MMP-9, PECAM-1, TGF α , TGF β 3, Tie-1, Tie-2, uPAR, VEGF, VEGF R2, VEGF R3, VEGF-D
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 μ l per array
Reproducibility	CV <20%
Assay duration	4 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 of 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 semi-quantitative cytokine antibody array, in which multiple cytokine antibodies are arrayed on solid support (membrane or glass slide). Detection of multiple cytokines is achieved through a sandwich-like ELISA procedure. Our current RayBio[®] Human Cytokine Antibody Array C or G series 2000 enables scientists to detect 174 human cytokines in a single experiment rapidly and inexpensively. The array data can be further validated and quantified by using RayBiotech ELISA kits.

Our new multiplex Quantibody[®] Array is another quantum leap forward in protein microarray technology. This glass-chip-based multiplexed sandwich ELISA system enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection specificity / sensitivity 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

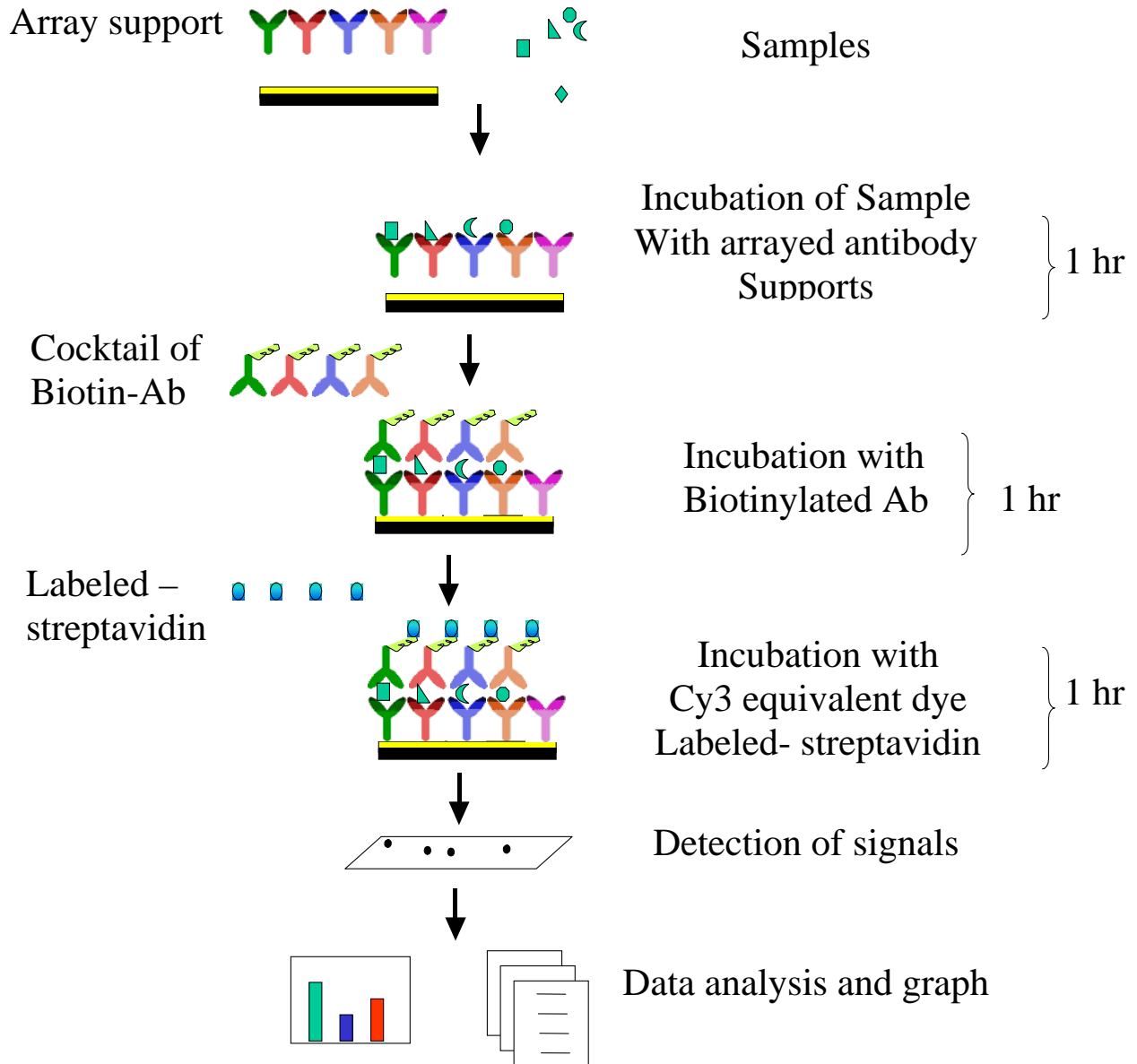
added, which can recognize a different isotope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled cy3 equivalent dye using a laser scanner. Unlike the traditional ELISA, Quantibody products use array format. By arraying 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 and negative control 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, were provided to generate a five-point standard curve of 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 unknown cytokine concentration in the samples will be determined.

Quantibody® Human Angiogenesis Array 1000 is the combination of Quantibody® Human Angiogenesis Arrays 2 and 3, where each kit can independently detect 30 distinct human cytokines. With Quantibody® Human Angiogenesis Array 1000, researchers can now measure the concentration of 60 different angiogenesis factors in a single experiment from as little as 100 µl samples. This is by far one of the most efficient products on the market for cytokine quantification.

Quantibody® array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for drug and biomarker discovery.

How it works



II. Materials Provided

Upon receipt, all the 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 chip, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at -20°C and all other components should be stored at 4°C. The entire kit should be used within 6 months of purchase.

Components*

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

* There are two independent sets of reagents for Quantibody® human angiogenesis arrays 2 and 3. 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 both 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 or cell culture supernatant or 20-200 µg of protein for cell lysates and tissue lysates.

If you experience high background or the readings exceed the detection range, further dilution 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.
- Avoid breaking glass slide.
- Handle glass chip in clean environment.

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.
- Avoid cross-contamination from overflowing solution to neighboring wells.
- 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 at 4⁰C for overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.

IV. Protocol

*Note: There are two sets of reagents for **two 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. Complete air dry the glass chip

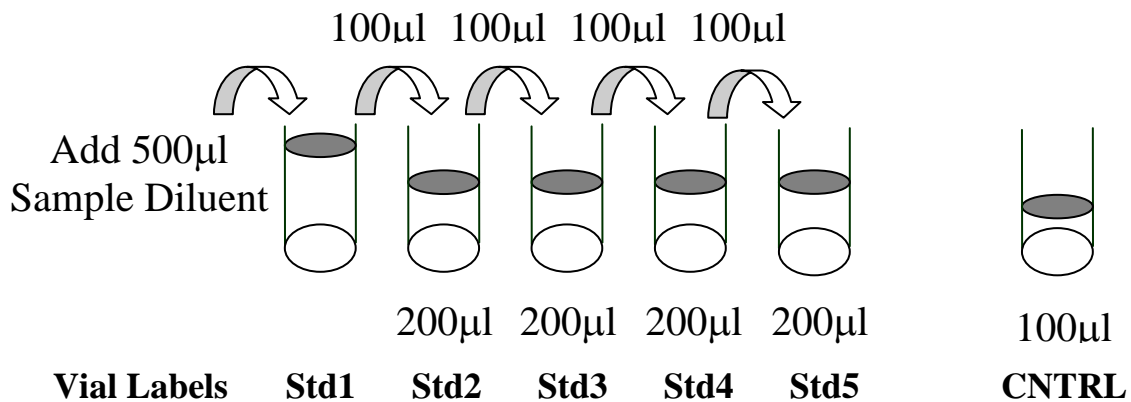
1. Take out the glass chip from the box; remove it from the plastic bag; peel off the covering film, and let it air dry at room temperature for at least 1-2 hours.

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. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1.

3. Label 4 clean microcentrifuge tubes as Std 2 to Std 5. Add 200µl Sample Diluent to each of the tubes.
4. Pipette 100µl Std1 into tube Std2 and mix gently. Perform 3 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 sample to the CNTRL tube, which will be used as negative control.

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 hour. (*Be careful to use the corresponding cytokine standard for the matching glass slide.*)

Note: The sample volume can be 50-100 µl. If sample volume is less than 70 µl, cover the gasket with adhesive sealer to prevent evaporation during incubation. Incubation may be done at 4⁰C for overnight.

*Note: We recommend using 50 to 100 µl of original or diluted serum, plasma or conditioned media or 20-200 µg of protein for cell lysates and tissue lysates. **In order to minimize the matrix effects and to lower the background of the assay, we recommended that the samples at least diluted 2 folds with Sample Diluent. Dilute the lysate at least 5 folds with Sample Diluent to make a total volume of 50 to 100 µl. Make sure there is no bubble in the wells.***

Note: The amount of sample used depends on the abundance of cytokines. More samples can be used if signals are too weak. If signals are too strong, the sample can be diluted further.

8. Decant the samples from each well, and wash 5 times with 200 µl of 1x Wash Buffer I and then 2 times with 200 µl of 1x Wash Buffer II at

room temperature with gentle shaking. Completely remove wash buffer in each wash step.

Note: avoid solution flowing into neighboring wells.

9. Reconstitute the Detection Antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly. (Be careful to use the corresponding detection antibody for the matching glass slide.)

Note: the diluted Detection antibodies can be stored at 4⁰C for 2-3 days.

10. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1 hour.

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

11. Wash as directed in step 8.

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.

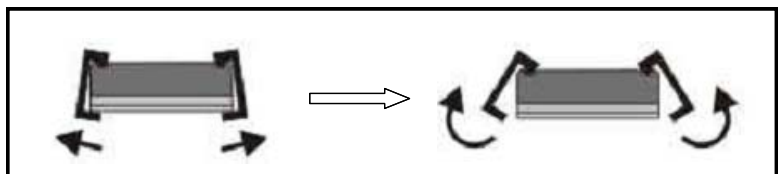
Note: incubation may be done at 4⁰C for overnight.

14. Wash four times with 1x Wash Buffer I.

D. Fluorescence Detection

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

Note: Be careful not to touch the surface of the array side



16. Place the slide in the slide washer (50 ml centrifuge tube), add enough 1x Wash Buffer I (about 40 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 40 ml) with gentle, and gently shake at room temperature for 5 minutes.

Note: This step can be done using slide chamber.

17. Decant Wash Buffer II and remove water droplets by centrifuging at 1,000 rpm for 3 minutes without cap.

Note: After the rinse step, proceed immediately for the drying step to prevent the deposit of the watermarks on the slide.

18. The signals can be visualized through use of a laser scanner equipped with a cy3 wavelength such as Axon GenePix. The settings should be: Excitation: 555 nm; Emission: 565 nm; Resolution: 10 um. Make sure that the signal from the standard well containing the highest concentration (Std1) receives the highest possible reading, yet remains unsaturated. Saved the image as a high resolution (16-bit) .tif file.

Note: In case the signal intensity for different cytokine varies greatly in the same array, we recommend using multiple scans for the low signal ones.

Note: we recommend scanning slide right after experiment. You can also store the slide at 4⁰C in a dry dark container for several days. If you do not have a laser scanner, RayBiotech can provide service for you. Just simply send your slide to us and we will take care of it.

E. 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 RayBio[®] Q Analyzer software is available. It gives visual output as well as digital value. More information can be found in section VIII.

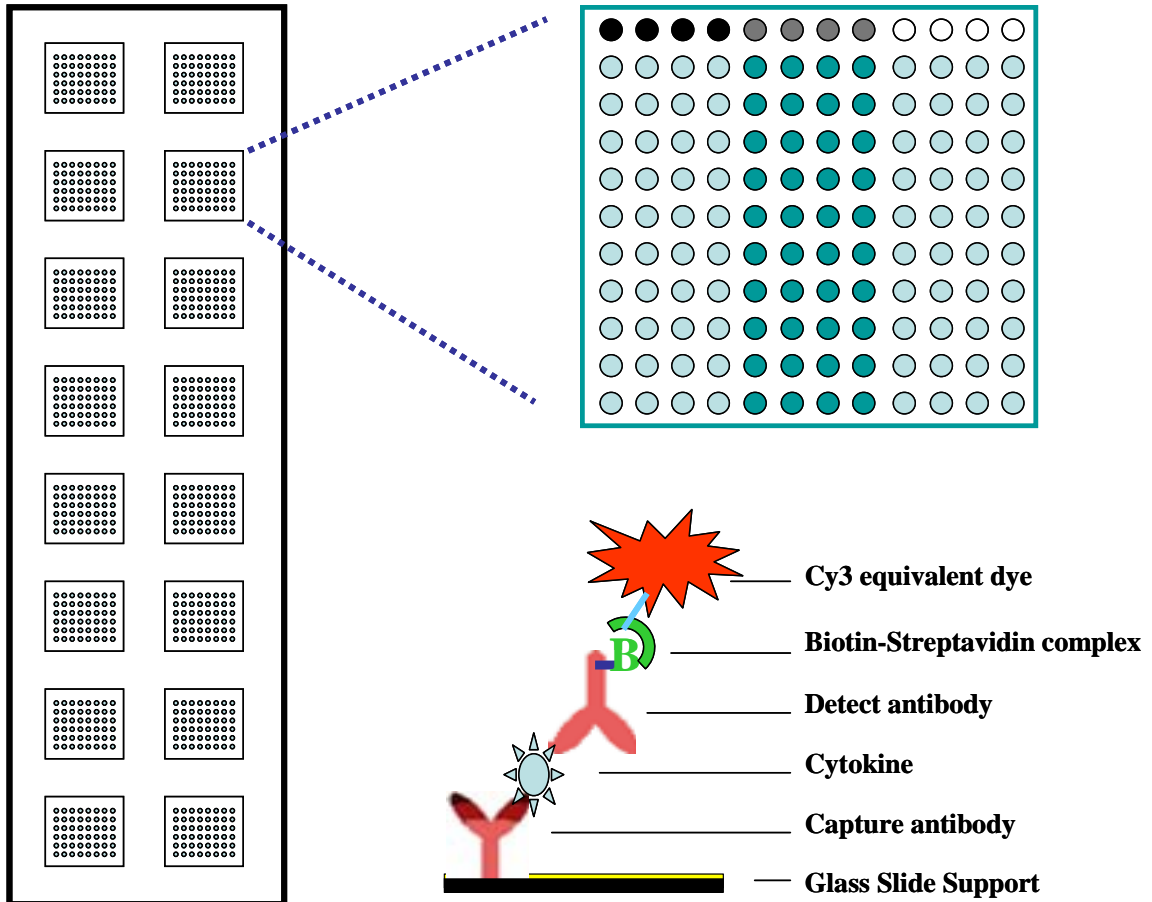
V. Cytokine array map

QAH-ANG-2

	1,2,3,4	5,6,7,8	9,10,11,12
<i>a</i>	POS1	POS2	NEG
<i>b</i>	Activin A	AgRP	Angiogenin
<i>c</i>	ANG-2	ANGPTL4	bFGF
<i>d</i>	ENA-78	GRO	HB-EGF
<i>e</i>	HGF	IFN γ	IGF-I
<i>f</i>	IL-1 α	IL-2	IL-6
<i>g</i>	IL-8	IL-17	IP-10
<i>h</i>	Leptin	LIF	MCP-1
<i>i</i>	PDGF-BB	PIGF	RANTES
<i>j</i>	TGF β 1	TIMP-1	TIMP-2
<i>k</i>	TNF α	TNF β	TPO

QAH-ANG-3

	1,2,3,4	5,6,7,8	9,10,11,12
<i>a</i>	POS1	POS2	NEG
<i>b</i>	ANG-1	Angiostatin	CXCL16
<i>c</i>	EGF	FGF-4	Follistatin
<i>d</i>	G-CSF	GM-CSF	I-309
<i>e</i>	IL-1 β	IL-4	IL-10
<i>f</i>	IL-12p40	IL-12p70	I-TAC
<i>g</i>	MCP-2	MCP-3	MCP-4
<i>h</i>	MMP-1	MMP-9	PECAM-1
<i>i</i>	TGF α	TGF β 3	Tie-1
<i>j</i>	Tie-2	uPAR	VEGF
<i>k</i>	VEGF R2	VEGF R3	VEGF-D



VI. Six-point cytokine standard curves and detection sensitivity

The five-point cytokine concentration used for generating the standard curve of a given antigen was listed below. The sensitivity of each protein, which is defined as the corresponding concentration at two standard deviations above the average fluorescence of 20 replicates of the negative control (0 pg/ml), is listed at the following table.

QAH-ANG-2 Standards and Sensitivity (pg/ml)

	CNTRL	Std5	Std4	Std3	Std2	Std1	Average	Stdev	LOD (pg/ml)
Activin A	0	1,235	3,704	11,111	33,333	100,000	56	6	344
AgRP	0	62	185	556	1,667	5,000	17	4	17
Angiogenin	0	247	741	2,222	6,667	20,000	31	12	15
ANG-2	0	99	296	889	2,667	8,000	62	4	35
ANGPTL4	0	2,469	7,407	22,222	66,667	200,000	29	3	1,768
bFGF	0	123	370	1,111	3,333	10,000	57	5	52
ENA-78	0	123	370	1,111	3,333	10,000	58	5	42
GRO	0	49	148	444	1,333	4,000	94	6	17
HB-EGF	0	49	148	444	1,333	4,000	21	3	21
HGF	0	49	148	444	1,333	4,000	40	13	12
IFN γ	0	25	74	222	667	2,000	36	6	14
IGF-I	0	617	1,852	5,556	16,667	50,000	88	9	424
IL-1 α	0	25	74	222	667	2,000	43	4	19
IL-2	0	49	148	444	1,333	4,000	48	4	29
IL-6	0	25	74	222	667	2,000	95	5	10
IL-8	0	12	37	111	333	1,000	113	8	2
IL-17	0	49	148	444	1,333	4,000	46	12	20
IP-10	0	25	74	222	667	2,000	116	7	20
Leptin	0	494	1,481	4,444	13,333	40,000	39	14	30
LIF	0	370	1,111	3,333	10,000	30,000	56	11	62
MCP-1	0	25	74	222	667	2,000	106	7	11
PDGF-BB	0	25	74	222	667	2,000	52	14	8
PIGF	0	49	148	444	1,333	4,000	35	16	6
RANTES	0	247	741	2,222	6,667	20,000	35	5	5
TGF β 1	0	1,235	3,704	11,111	33,333	100,000	155	11	1,221
TIMP-1	0	494	1,481	4,444	13,333	40,000	15	3	91
TIMP-2	0	247	741	2,222	6,667	20,000	27	6	38
TNF α	0	25	74	222	667	2,000	88	11	12
TNF β	0	123	370	1,111	3,333	10,000	60	6	59
TPO	0	2,469	7,407	22,222	66,667	200,000	19	6	1,060
TNF sRII	0	494	1,481	4,444	13,333	40,000	28	34	70

QAH-ANG-3 Standards and Sensitivity (pg/ml)

	CNTRL	Std5	Std4	Std3	Std2	Std1	Average	Stdev	LOD (pg/ml)
ANG-1	0	617	1,852	5,556	16,667	50,000	198	11	228
Angiostatin	0	24,691	74,074	222,222	666,667	2,000,000	135	9	2,722
CXCL16	0	25	74	222	667	2,000	168	14	13
EGF	0	1	4	11	33	100	124	4	1
FGF-4	0	1,235	3,704	11,111	33,333	100,000	152	6	843
Follistatin	0	1,235	3,704	11,111	33,333	100,000	101	6	767
G-CSF	0	123	370	1,111	3,333	10,000	31	4	67
GM-CSF	0	25	74	222	667	2,000	70	11	7
I-309	0	49	148	444	1,333	4,000	28	5	13
IL-1 β	0	12	37	111	333	1,000	68	7	4
IL-4	0	12	37	111	333	1,000	171	17	6
IL-10	0	12	37	111	333	1,000	89	4	13
IL-12p40	0	123	370	1,111	3,333	10,000	46	6	54
IL-12p70	0	6	19	56	167	500	63	6	2
I-TAC	0	25	74	222	667	2,000	146	6	10
MCP-2	0	12	37	111	333	1,000	60	5	8
MCP-3	0	49	148	444	1,333	4,000	45	8	10
MCP-4	0	494	1,481	4,444	13,333	40,000	20	4	119
MMP-1	0	247	741	2,222	6,667	20,000	190	8	172
MMP-9	0	247	741	2,222	6,667	20,000	15	4	110
PECAM-1	0	247	741	2,222	6,667	20,000	62	4	103
TGF α	0	123	370	1,111	3,333	10,000	7	7	25
TGF β 3	0	494	1,481	4,444	13,333	40,000	31	2	482
Tie-1	0	6,173	18,519	55,556	166,667	500,000	40	5	2,842
Tie-2	0	247	741	2,222	6,667	20,000	33	4	145
uPAR	0	494	1,481	4,444	13,333	40,000	145	13	171
VEGF	0	123	370	1,111	3,333	10,000	37	4	126
VEGF R2	0	123	370	1,111	3,333	10,000	82	5	43
VEGF R3	0	247	741	2,222	6,667	20,000	41	5	146
VEGF-D	0	494	1,481	4,444	13,333	40,000	29	3	109

VII. System Recovery

The recovery of the Human antigens by the kits were tested through spiking different levels of the recombinant proteins in 4x diluted Human serum H4522 and 4x diluted Human cell culture media (CM). The non-spiked serum sample and cell culture media were used as negative control. The recovery rate for each antigen was then determined by subtracting the endogenous antigen level from the observed value and divided by the spiking antigen concentration.

QAH-ANG-2 Serum and Cell Media Recovery

ID	Spiking	SA	SA+Ag	SA%	CM	CM+Ag	CM%
Activin A	50,000	0	41082	82.2%	0	37715	75.4%
AgRP	2,500	125	2985	114.4%	0	2627	105.1%
Angiogenin	10,000	over	over	-	50	7700	76.5%
ANG-2	2,000	1800	3362	78.1%	0	1867	93.3%
ANGPTL4	100,000	19974	143712	123.7%	0	70907	70.9%
bFGF	5,000	0	3920	78.4%	0	4080	81.6%
ENA-78	2,500	14	2639	105.0%	0	2122	84.9%
GRO	2,000	179	1931	87.6%	10	2492	124.1%
HB-EGF	1,000	9	1096	108.7%	0	988	98.8%
HGF	2,000	34	1695	83.1%	3	1630	81.3%
IFN γ	2,000	18	1773	87.8%	7	1632	81.2%
IGF-I	25,000	948	21833	83.5%	0	17910	71.6%
IL-1 α	1,000	0	814	81.4%	0	897	89.7%
IL-2	2,000	0	1580	79.0%	0	1473	73.6%
IL-6	1,000	122	1181	105.9%	83	1214	113.1%
IL-8	500	1	363	72.4%	14	484	94.0%
IL-17	3,000	8	2943	97.8%	0	3313	110.4%
IP-10	1,000	44	961	91.7%	22	867	84.4%
Leptin	20,000	2013	28898	134.4%	0	19834	99.2%
LIF	15,000	48	17374	115.5%	0	13484	89.9%
MCP-1	1,000	129	856	72.6%	2441	3456	101.5%
PDGF-BB	2,000	304	1922	80.9%	1	1560	77.9%
PIGF	2,000	3	1662	83.0%	0	1519	75.9%
RANTES	20,000	500	18473	89.9%	0	18430	92.1%
TGF β 1	50,000	42761	86990	88.5%	0	34590	69.2%
TIMP-1	20,000	over	over	-	over	over	-
TIMP-2	10,000	over	over	-	13331	21154	78.2%
TNF α	1,000	31	1138	110.7%	0	1017	101.6%
TNF β	1,500	0	1306	87.1%	0	973	64.9%
TPO	100,000	1449	72128	70.7%	741	125311	124.6%

QAH-ANG-3 Serum and Cell Media Recovery

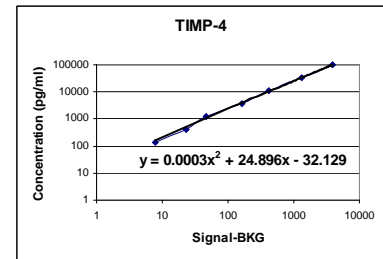
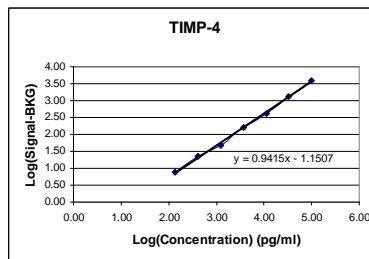
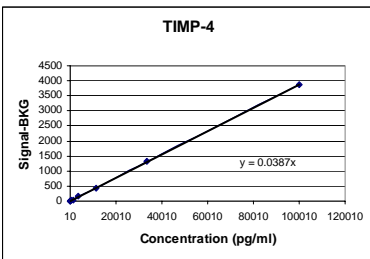
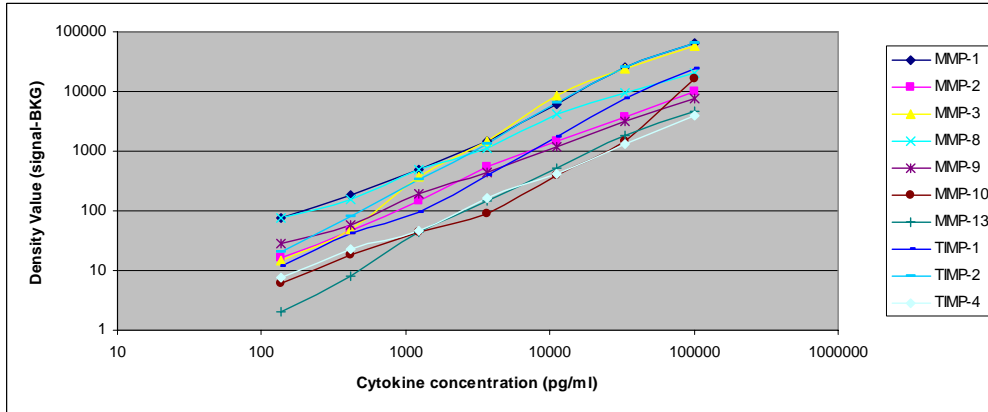
ID	Spiking	SA	SA+Ag	SA%	CM	CM+Ag	CM%
ANG-1	25000	9154.8	31374.0	88.9%	6.0	27090.1	108.3%
Angiostatin	1000000	1970046.0	3112128.7	114.2%	16122.7	931280.5	91.5%
CXCL16	1000	3640.4	4727.8	108.7%	6.2	1088.9	108.3%
EGF	50	3.7	38.8	70.1%	0.0	44.0	88.1%
FGF-4	50000	6758.0	57752.2	102.0%	0.0	42143.7	84.3%
Follistatin	50000	6597.8	44620.6	76.0%	0.0	60538.6	121.1%
G-CSF	5000	141.9	3798.4	73.1%	18.1	5362.6	106.9%
GM-CSF	1000	14.8	852.6	83.8%	0.0	1080.0	108.0%
I-309	2000	88.2	2338.3	112.5%	32.1	1831.6	90.0%
IL-1β	500	27.8	522.0	98.8%	6.1	419.3	82.7%
IL-4	500	44.9	586.3	108.3%	2.4	568.8	113.3%
IL-10	500	129.1	612.5	96.7%	0.0	556.6	111.3%
IL-12p40	5000	21.5	3916.8	77.9%	5.4	4698.1	93.9%
IL-12p70	250	16.5	284.7	107.3%	0.0	219.6	87.9%
I-TAC	1000	187.8	966.3	77.9%	0.0	1087.3	108.7%
MCP-2	500	17.5	508.4	98.2%	0.0	638.2	127.6%
MCP-3	2000	39.7	2273.7	111.7%	4.6	2237.3	111.6%
MCP-4	20000	14.9	22912.4	114.5%	6.2	20560.4	102.8%
MMP-1	10000	1050.6	9430.5	83.8%	0.0	10753.6	107.5%
MMP-9	5000	7898.5	12474.2	91.5%	60.3	4859.6	96.0%
PECAM-1	10000	974.5	9702.0	87.3%	2.9	8362.2	83.6%
TGFα	5000	7.6	6055.2	121.0%	13.6	5472.1	109.2%
TGFβ3	20000	2309.6	22793.1	102.4%	434.7	21549.5	105.6%
Tie-1	250000	12900.5	256689.5	97.5%	4828.3	247573.7	97.1%
Tie-2	10000	1290.6	9622.7	83.3%	581.5	9920.5	93.4%
uPAR	20000	3157.3	17410.8	71.3%	828.1	18571.3	88.7%
VEGF	5000	4339.3	9168.1	96.6%	1751.6	7486.6	114.7%
VEGF R2	5000	3533.1	7232.9	74.0%	206.9	5057.3	97.0%
VEGF R3	10000	235.4	6914.8	66.8%	69.3	8616.7	85.5%
VEGF-D	20000	469.0	21507.9	105.2%	530.1	24198.6	118.3%

VIII. 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 completely 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 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
	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.

VIII. Sample Raybio® Q Analyzer Output

Raybio® Q Analyzer greatly facilitates the data analysis. Instead of tedious calculation, user can now quickly figure out the unknown sample concentration through a simple copy and paste process. The program can automatically remove the outlier spots, and users can choose either linear regression or log-log algorithms to best meet their analytical needs.



Sample Cytokine Concentration (pg/ml) (Base on Linear Regression)								
ID	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
MMP-1	0	538	3,626	8,202	43,812	96,822	551	2,122
MMP-2	0	69	7,066	7,479	26,460	46,335	1,496	2,802
MMP-3	0	6	1,362	3,573	21,670	25,902	30,364	966
MMP-8	0	234	917	2,029	9,986	18,793	1,409	678
MMP-9	0	2,891	9,740	6,013	14,510	27,976	37,079	19,933
MMP-10	0	10,952	16,428	37,660	116,437	406,305	320,779	56,489
MMP-13	0	1,021	1,293	4,167	10,277	18,553	1,056	1,797
TIMP-1	0	1,356	1,111	2,759	6,923	19,838	71,685	9,304
TIMP-2	0	234	131	1,876	4,139	20,897	133,539	5,705
TIMP-4	0	2,288	5,917	8,094	25,384	46,641	16,342	3,912

IX. Reference List

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X. Experiment Record Form

Date: _____

File Name: _____

Laser Power: _____

PMT: _____

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

1	2
3	4
5	6

Note:

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