Quantibody® Human Cytokine Array 8

Quantitative measurement of 40 human cytokines

Catalog #: QAH-CYT-8

User Manual
Last revised July 18, 2019

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

ISO 13485 Certified

3607 Parkway Lane, Suite 100
Norcross, GA 30092
Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax:770-206-2393
Web: www.RayBiotech.com, Email: info@raybiotech.com

Table of Contents

Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (40)	ANGPTL3, beta IG-H3, CA9, Cathepsin B, CD23, CD26 (DPPIV), CHI3L1, Coagulation Factor III (Tissue Factor/Thromboplastin), CTLA-4 (CD152), Dkk-4, EDA-A2, Erythropoietin R, FGF-6, FGF-9, FOLH1 (PSMA), Gas 1, IGFBP-5, IL-38 (IL-1 F10), IL-36 Ra (IL-1 F5), IL-36 alpha (IL-1 F6), IL-37 (IL-1 F7), IL-36 beta (IL-1 F8), IL-36 gamma (IL-1 F9), IL-1 R5 (IL-18 R alpha), IL-10 R alpha, IL-17C, IL-18, IL-20, IL-34, IL-5 R alpha, Layilin, Leptin R, Marapsin, Mer, MMP-7, P-Cadherin, Prostasin, SIGIRR, TGF beta R3, TWEAK (TNFSF12) *See Section IX for Array Map*
Format	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
Detection Method	Fluorescence. Go to www.RayBiotech.com/Scanners for a list of compatible laser scanners.
Sample Volume	50 - 100 µl per array
Reproducibility	CV <20%
Assay Duration	6 hours

II. Introduction

Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in interactions between different cell types, cellular responses to environmental conditions, and maintenance of homeostasis. In addition, cytokines are also involved in most disease processes, including cancer and cardiac diseases.

The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent assay (ELISA). In this method, target protein is immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the enzyme complex can then be visualized through the use of a substrate that produces a detectable signal. While this traditional method works well for a single protein, the overall procedure is time consuming and
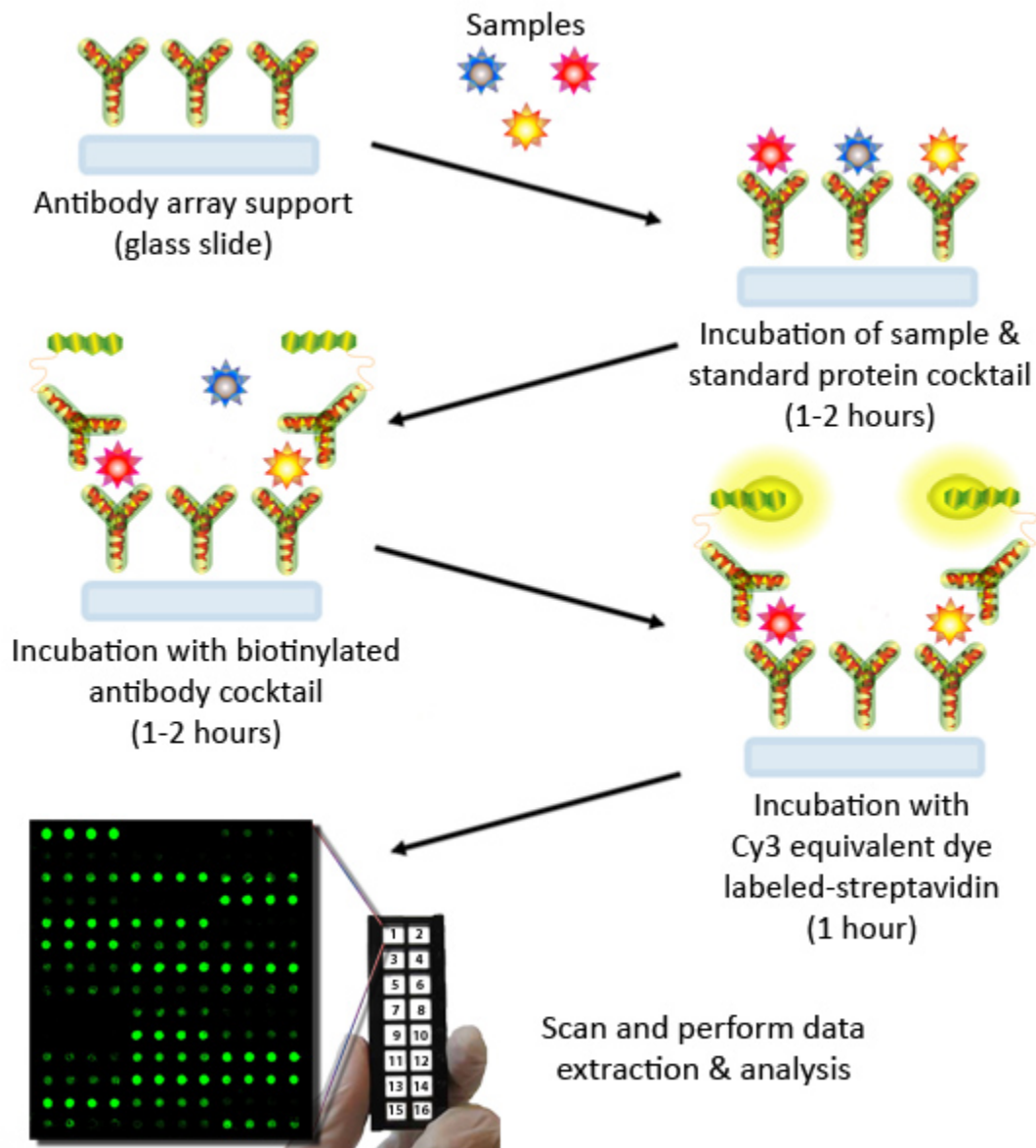
requires a relatively high volume of sample. Thus, conservation of precious small sample quantities becomes a challenging task. Innovations in microarray technology over the last decade have addressed this problem. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which have now been widely applied in the research community with hundreds of peer reviewed publications, including top-tier journals such as *Cell* and *Nature*.

The Quantibody® array, our multiplexed sandwich ELISA-based quantitative array platform, enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and the high throughput of arrays. Like a traditional sandwich-based ELISA, it uses a pair of cytokine specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target cytokine is trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different epitope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-conjugated Cy3 equivalent dye, using a laser scanner. Unlike the traditional ELISA, Quantibody products use an array format. By arraying multiple cytokine specific capture antibodies onto a glass support, quantitative, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is divided into 16 wells of identical cytokine antibody arrays. Each antibody, together with the positive controls is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples on one slide. Four slides can be nested into a tray, which matches a standard microplate footprint and allows for automated robotic high throughput process of 64 arrays simultaneously. For cytokine quantification, the array specific cytokine standards, whose concentration has been predetermined, are provided to generate a standard curve for each cytokine. In a real experiment, standard cytokines and samples will be assayed in each array simultaneously through a sandwich ELISA procedure. By comparing signals from unknown samples to the standard curve, the cytokine concentration in the samples will be determined.

Quantibody® array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Our current high density Quantibody kits allow scientists to quantitatively determine the concentration of 1000 human, 200 mouse, and 67 rat cytokines in a single experiment. This is not only one of the most efficient products on the market for cytokine quantification, but makes it more affordable for quantification of large number of proteins. Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for drug and biomarker discovery.

III. How It Works

Antibody array support
(glass slide)

Samples

Incubation of sample &
standard protein cocktail
(1-2 hours)

Incubation with biotinylated
antibody cocktail
(1-2 hours)

Incubation with
Cy3 equivalent dye
labeled-streptavidin
(1 hour)

Scan and perform data
extraction & analysis

IV. Materials Provided

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	QAH-CYT-8 S	Human Cytokine Array 8 Glass Slide	1	2
2	QA-SDB	Quantibody® Sample Diluent	15 ml	
3	AA-WB1-30ML	20X Wash Buffer I	2 x 30 ml	3 x 30 ml
4	AA-WB2-30ML	20X Wash Buffer II	30 ml	
5	QAH-CYT-8 -STD	Human Cytokine Array 8 Lyophilized Standard Mix**	1 Vial	
6	QAH-CYT-8 B	Human Cytokine Array 8 Biotinylated Antibody Cocktail	1-25 µl	2 x 1-25 µl
7	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 µl	2 x 5 µl
8	QA-SWD	Slide Washer/Dryer	1 x 30 ml Tube	
9	QA-ADH	Adhesive Film	1	2

* 4 slide kits are comprised of 2 separate 2 slide kits.
** See Section X for detailed cytokine concentrations after reconstitution.

V. Storage

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

VI. Additional Materials Required

- Benchtop rocker or orbital rocker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- 1.5 ml Polypropylene microcentrifuge tubes

VII. General Considerations

A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contains cytokines.
- Each array needs 100 µl of total sample volume. To avoid matrix effects, we recommend using a minimum of 2-fold sample dilution of culture media, body fluids, or 0.5-1mg/ml total protein for lysates, after a 5-fold to 10-fold dilution to minimize the effects of any detergent(s). Please be aware, more sample volume is required for combination arrays. For example, the minimum sample volume for a 10-array kit is 500 µl, or 500 µg lysate.
- The suggested serum/plasma dilution for this array is: 2x

B. Handling Glass Slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with powder free gloves.
- Handle glass slide/s in clean environment.
- Permanent marker ink can significantly interfere with fluorescent signal detection. To help distinguish one slide from another, you may make a small marking (such as a number or a star) along the top or bottom edge, using a green or blue ultra-fine point Sharpie$^{®}$ brand marker. This can also serve to orient the slide. For best results during scanning, please **DO NOT**:
  - Write anywhere on the front (arrayed) side of the slide
  - Write on the slide while it is wet
  - Use red or black colored ink anywhere on the slide
  - Write over the arrayed well areas of the slide, as this interferes with scanning.

C. Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rocking or rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µl of sample or reagent is used.

- Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.
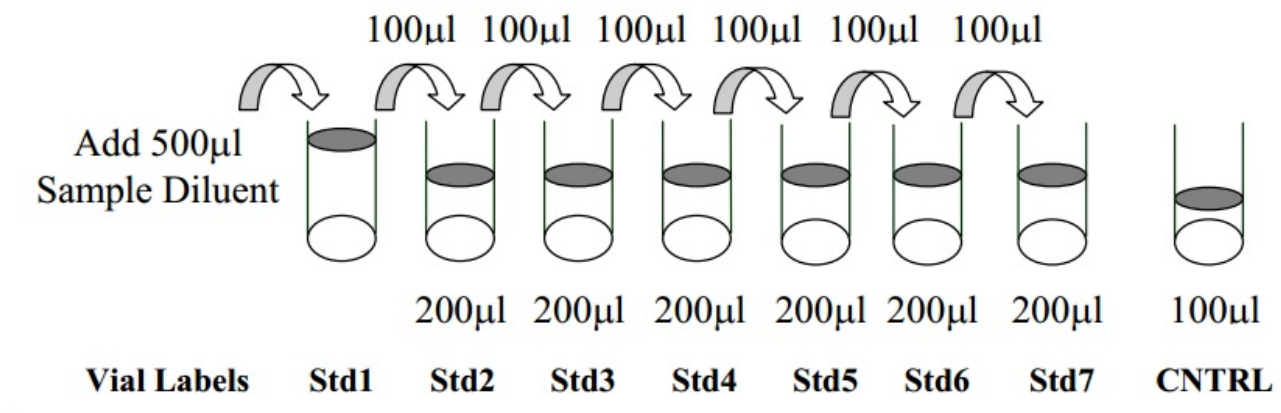
VIII. Protocol

A. Completely Air Dry The Glass Slide

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

Incomplete drying of slides before use may cause the formation of "comet tails," thin directional smearing of antibody spots.

B. Prepare Cytokine Standard Dilutions

There is only one vial of standard provided in the two-slide kit, which is enough for making two standard curves. Reconstitute the lyophilized standard within one hour of usage. If you must use the standard for two different days, store only the Std1 dilution at -80°C.

	100μl	100μl	100μl	100μl	100μl	100μl	
Add 500μl Sample Diluent							
		200μl	200μl	200μl	200μl	200μl	200μl	100μl
Vial Labels	Std1	Std2	Std3	Std4	Std5	Std6	Std7	CNTRL

2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500 μl Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1.

3. Label 6 clean microcentrifuge tubes as Std2 to Std7. Add 200 µl Sample Diluent to each of the tubes.

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

5. Add 100 µl Sample Diluent to another tube labeled as CNTRL. Do not add standard cytokines or samples to the CNTRL tube, which will be used as negative control. For best results, include a set of standards in each slide.

Since the starting concentration of each cytokine is different, the serial concentrations from Std1 to Std7 for each cytokine are varied which can be found in Section X.

C. Blocking & Incubation

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

7. Decant buffer from each well. Add 100 µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour.

Longer incubation time is preferable for higher signals. This step may be done overnight at 4°C.

We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 250 µg/ml-1 mg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation, especially if less than 70 ul of sample or reagent is used.

8. Wash:

   • Decant the samples from each well, and wash 5 times (5 min each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with H2O.

   • *(Optional for Cell and Tissue Lysates)* Put the glass slide with frame into a box with 1X Wash Buffer I (cover the whole glass slide and frame with Wash Buffer

I), and wash at room temperature with gentle rocking for 20 min.

- Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 150 µl of 1X Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step. Dilute 20X Wash Buffer II with H2O.

Incomplete removal of the wash buffer in each wash step may cause "dark spots," the background signals higher than the spots.

D. Incubation with Biotinylated Antibody Cocktail & Wash

9. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.

10. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour.

Longer incubation time is preferable for higher signals and backgrounds

11. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I and then 2 times with 150 µl of 1x Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

E. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash

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

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

14. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

F. Fluorescence Detection

15. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.
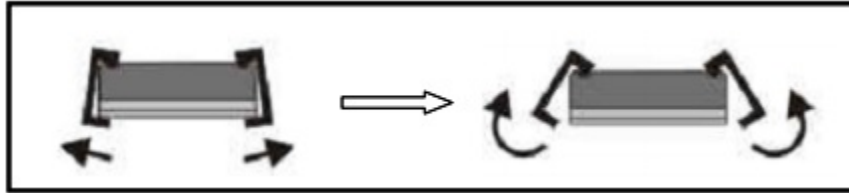
Be careful not to touch the surface of the array side.

16. Place the slide in the Slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) and gently shake at room temperature for 5 minutes.

17. Remove water droplets completely by gently applying suction with a pipette to remove water droplets. Do not touch the array, only the sides.

    You may also dry the glass slide by a compressed N2 stream.

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

In case the signal intensity for different cytokine varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal cytokines, and a low PMT for high signal cytokines.

G. Data Analysis

19. Data extraction can be done using the GAL file that is specific for this array along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). GAL files can be found here: www.RayBiotech.com/Gal-Files.html.

    Need help analyzing all that data? Copy and paste your data into the Q-Analyzer Tool specific for this array, catalog number: **QAH-CYT-8-SW**. More information can be found in Section XII.

Experiment

Image Scan
A list of compatible
laser scanners can be
found here:
RayBiotech.com/Scanners

Data Extraction
(GenePix, etc.)

Data Computation
Q-Analyzer software
sold separately
(see section VIII.)

Analyze Results
(pg/ml concentrations)
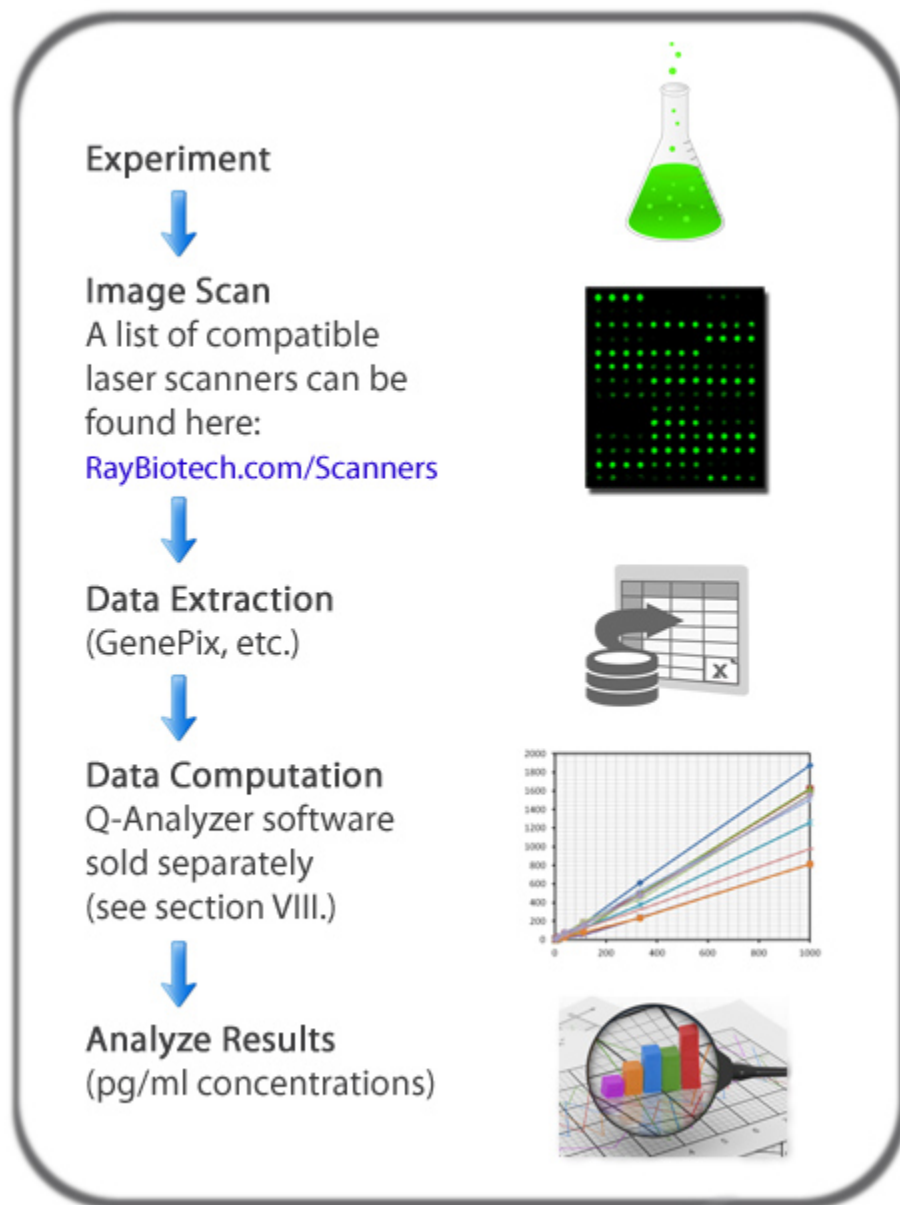
IX. Array Map & Standard Curves

	1	2	3	4	1	2	3	4	1	2	3	4
	header: (hCYT-8 Map) Each antibody is printed in quadruplicate horizontally											

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ANGPTL3			
B	beta IG-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 R alpha				IL-10 R alpha			
K	Layilin				Leptin R				Marapsin			
L	Mer				MMP-7				P-Cadherin			
M	Prostasin				PSMA				SIGIRR			
N	TGF beta RIII				Tissue Factor (TF)				TWEAK			

QAH-CYT-8 Standard Curves
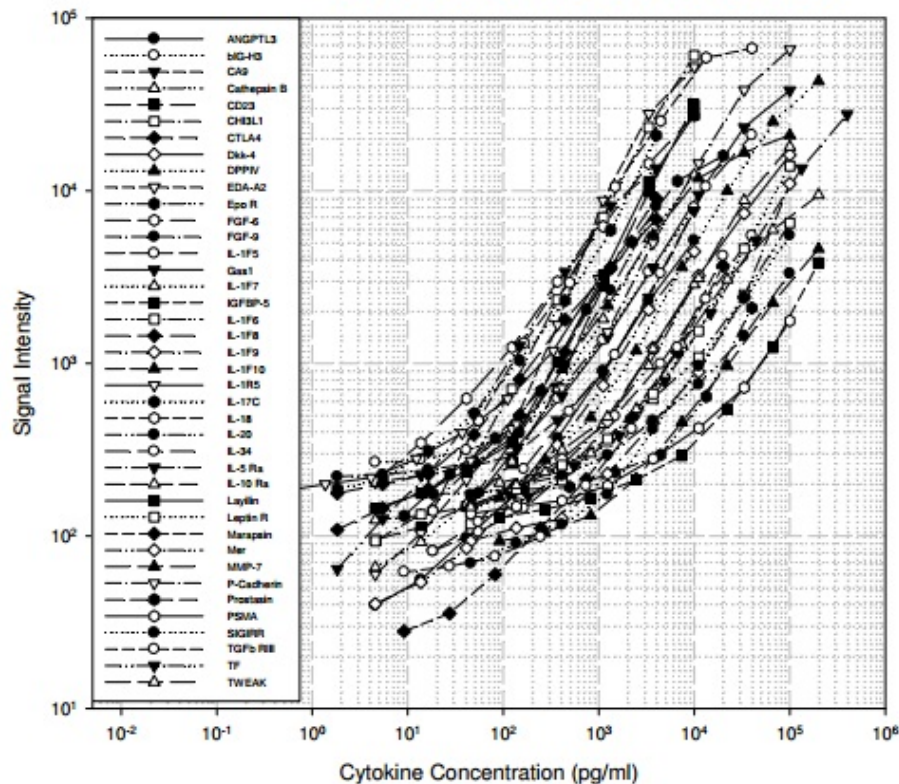
X. Standard Concentrations

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

Serial standard concentration (pg/ml)

	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
ANGPTL3	0	14	41	123	370	1,111	3,333	10,000
βIG-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 Rα	0	549	1,646	4,938	14,815	44,444	133,333	400,000
IL-10 Rα	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
TGFβ 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

XI. Spiking & Recovery

The antibody pairs used in the kit have been tested to recognize their specific antigen. The spiking recovery rates of each protein in some common fluids are listed in the following tables.

The spiking recovery rate for human culture media and serum

(pg/ml)	Spiking	CM	CM+Ag	CM%	Serum	Serum+Ag	Serum%
ANGPTL3	5,000	10	10,584	211%	8,843	12,758	78%
βIG-H3	5,000	5,589	9,083	70%	4,374	7,120	55%
CA9	5,000	81	4,018	79%	0	4,002	80%
Cathepsin B	10,000	923	9,152	82%	818	10,639	98%
CD23	5,000	0	3,797	76%	143	2,855	54%
CHI3L1	5,000	3,631	7,366	75%	6,197	5,744	-
CTLA4	2,000	0	2,447	122%	0	1,004	50%
Dkk-4	50,000	0	52,429	105%	209	30,514	61%
DPPIV	100,000	39	80,750	81%	43,683	45,822	2%
EDA-A2	5,000	0	4,496	90%	0	4,200	84%
Epo R	20,000	0	18,782	94%	0	17,157	86%
FGF-6	5,000	0	4,338	87%	0	4,166	83%
FGF-9	2,000	0	1,802	90%	0	1,032	52%
Gas1	50,000	0	64,600	129%	0	48,587	97%
IGFBP-5	100,000	863	108,549	108%	0	110,436	110%
IL-1F5	200,000	0	233,801	117%	17,572	174,906	79%
IL-1F6	50,000	0	47,472	95%	0	50,807	102%
IL-1F7	50,000	0	53,759	108%	146	22,684	45%
IL-1F8	2,000	0	2,058	103%	0	1,295	65%
IL-1F9	50,000	0	42,475	85%	0	27,087	54%
IL-1F10	100,000	0	141,759	142%	2,160	131,237	129%
IL-1R5	500	0	687	137%	0	913	183%
IL-17C	200,000	1,759	202,598	100%	0	93,568	47%
IL-18	20,000	0	24,192	121%	74	8,870	44%
IL-20	100,000	2,145	93,449	91%	0	142,762	143%
IL-34	20,000	0	8,434	42%	0	12,508	63%
IL-5 Rα	200,000	21	186,405	93%	0	126,640	63%
IL-10 Rα	100,000	776	114,978	114%	0	112,518	113%
Layilin	5,000	46	6,780	135%	0	1,845	37%
Leptin R	50,000	88	36,379	73%	0	32,201	64%
Marapsin	20,000	0	17,315	87%	2,474	29,551	135%
Mer	5,000	0	8,723	174%	0	7,598	152%
MMP-7	50,000	1,554	70,836	139%	973	52,519	103%
P-Cadherin	50,000	741	60,633	120%	27,552	69,308	84%
Prostasin	10,000	32	5,868	58%	0	5,576	56%
PSMA	100,000	1,234	139,781	139%	0	159,932	160%
SIGIRR	50,000	0	59,392	119%	0	24,474	49%
TGFβ RIII	10,000	38	14,171	141%	4,155	19,112	150%
TF	2,500	18	3,168	126%	40	3,476	137%
TWEAK	50,000	91	57,724	115%	1,939	42,169	80%

XII. Quantibody® Q-Analyzer

The Q-Analyzer is an array specific, Excel-based program. It is much more than a simple calculation macro; it performs sophisticated data analysis (see below for description).

The Q-Analyzer Tool specific for this array is catalog number: **QAH-CYT-8-SW**.

Key features:

- Simplicity: Easy to operate and requires no professional training. With a simple copy and paste process, the cytokine concentration is determined.

- Outlier Marking & Removing: The software can automatically mark and remove the outlier spots for more accurate data analysis

- Normalization: The program allows for intra- and inter-slide normalization for large numbers of samples.

- Two Positive Controls: The program utilizes the two positive controls in each array for normalization.

- Two Analytical Algorithms: Users can choose either linear regression or log-log algorithms to meet their analytical needs.

- Two Data Outputs: standard curves and digital concentration.

- User Intervention: The program allows for user manual handling of outliers and other analytical data.

- Lower and Upper Limits Determination: The program automatically marks out the values below or above the detection range.

- Standard Deviation: The program outputs the standard deviations of the quadruplicate spots for data accuracy.

- Analytical Tips: Q-Analyzer analysis tips are included in the program.

XIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Increase incubation time or change sample incubation step to overnight
	Too low protein concentration in sample	Lessen dilution or do not dilute sample. Concentrate sample if necessary.
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Decrease amount of rocking during incubations. check for bubble formation and remove bubbles.
	Arrays are not completed covered by reagent	Completely cover arrays with solution for all required steps.
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer and other solutions into neighboring wells.
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard well at the room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions.
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated.
	Use freeze-thawed cytokine standards	Always use new cytokine standard vial for new set of experiment. Discard any leftover.
High background	Overexposure	Lower the PMT or signal gain.
	Dark spots	Completely remove wash buffer in each wash step.
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Work in clean environment
	Slide is allowed to dry out	Don't dry out slides during experiment.

XIV. Select Quantibody® Publications

1. Zeng Q., et al. The functional behavior of a macrophage/fibroblast co-culture model derived from normal and diabetic mice with a marine gelatin-oxidized alginate hydrogel. Biomaterials. 2010 Aug;31(22):5772-81. doi: 10.1016/j.biomaterials.2010.04.022.
   Species: Mouse

2. Toh H, Wang W, Chia W, Kvistborg P, Sun Li,et al. Clinical Benefit of Allogeneic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients.Clin Cancer Res. 2009;15(24):7726-7736
   Species: Human
   Sample Type: Plasma

3. Du Y, Wei X, He Y, Wei G, Hampel H, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. Alzheimer Dementia. 2008;4(4 Suppl):T484 (Abstract P2-380).
   Species: Human
   Sample Type: Plasma

4. Jonnalagadda D., et al. Platelet secretion is kinetically heterogeneous in an agonist-responsive manner. December 20, 2012; Blood: 120 (26). http://dx.doi.org/10.1182/blood-2012-07-445080
   Species: Human
   Sample Type: Conditioned Media

5. Vargas-Inchaustegui D., Hogg A., Tulliano G., et al.CXCL10 Production by Human Monocytes in Response to Leishmania braziliensis Infection. Infect. Immun. January 2010 vol. 78 no. 1 301-308
   Species: Human
   Sample Type: Serum

6. Zhai Y, Zhong Z, Chen C-YA, Xia Z, Song L, Blackburn MR, Shyu A-B. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. Mol Cell Biol. 2008; 28(24):7414-7426.
   Species: Human

7. Huggenberger R., et al. Stimulation of lymphangiogenesis via VEGFR-3 inhibits chronic skin inflammation. J Exp Med. 2010 Sep 27;207(10):2255-69. doi: 10.1084/jem.20100559.
   Species: Mouse
   Sample Type: Tissue Lysate

8. Jurk D., Wilson C., Passos J., et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nature Communications 2, Article number: 4172. doi:10.1038/ncomms5172
   Species: Mouse
   Sample Type: Conditioned Media

9. Bethunaickan, R., Sahu, R., Liu, Z., Tang, Y. T., Huang, W., Edegbe, O., Tao, H., Ramanujam, M., Madaio, M. P. and Davidson, A. (2012), Anti-tumor necrosis factor alpha treatment of interferon-alpha-induced murine lupus nephritis reduces the renal macrophage response but does not alter glomerular immune complex formation. Arthritis & Rheumatism, 64: 3399-3408. doi: 10.1002/art.34553
   Species: Mouse
   Sample Type: Tissue Lysate

10. Hou T., Li Z., Luo F., Xie Z., Wu X., Xing J., Dong S., Xu J. A composite demineralized bone matrix e Self assembling peptide scaffold for enhancing cell and growth factor activity in bone marrow. Biomaterials, Available online 19 April 2014. [Epub ahead of print]
    Species: Mouse
    Sample Type: Tissue Lysate

11. Feng W., Madajka M., Kerr B., Mahabeleshwar G., White S., Byzova T. A novel role for platelet secretion in angiogenesis: mediating bone marrow-derived cell mobilization and homing. Blood April 7, 2011 vol. 117 no. 14 3893-3902
    Species: Mouse

XV. Experiment Record Form

Date:_____

File Name:_____

Laser Power:_____

PMT:_____

Well No.	Sample Name	Dilution factor
1	CNTRL	
2	Std7	
3	Std6	
4	Std5	
5	Std4	
6	Std3	
7	Std2	
8	Std1	
9		
10		
11		
12		
13		
14		
15		
16		

XVI. How to Choose a Quantibody® Array?

Species-based selection:

Human (QAH-)	Mouse (QAM-)	Rat (QAR-)	Bovine (QAB-)	Canine (QAC-)
Equine (QAE-)	Feline (QAF-)	Primates (QAN-)	Porcine (QAP-)	Rabbit (QAL-)

Function-based selection:

Adhesion Molecule Arrays	Angiogenesis Arrays	Bone Metabolism Arrays	Chemokine Arrays
Custom Arrays	Cytokine Arrays	Growth Factor Arrays	IGF Signaling Arrays
IL-1 Family Arrays	Immune Response Arrays	Inflammation Arrays	Interleukin Arrays
Isotyping Arrays	MMP Arrays	Obesity Arrays	Ophthalmic Arrays
Periodontal Disease Arrays	Receptor Arrays	Th1/Th2/Th17 Arrays	

Cytokine Number-based selection:

Arrays are available in the Quantibody® platform to detect 1000 human, 200 mouse, or 67 rat proteins. GLP-Compliant testing services are also available.

To learn more about the Quantibody® Antibody Array, visit
www.RayBiotech.com/Quantibody-Multiplex-Elisa-Array.html

Quantibody® is the trademark of RayBiotech, Inc.
This product is for research use only.

©2019 RayBiotech, Inc