

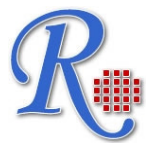
# Quantibody<sup>®</sup> Porcine Cytokine Array 2

--Quantitative measurement of 10 porcine cytokines

Patent Pending Technology

User Manual (Version July 2013)

Cat # QAP-CYT-2



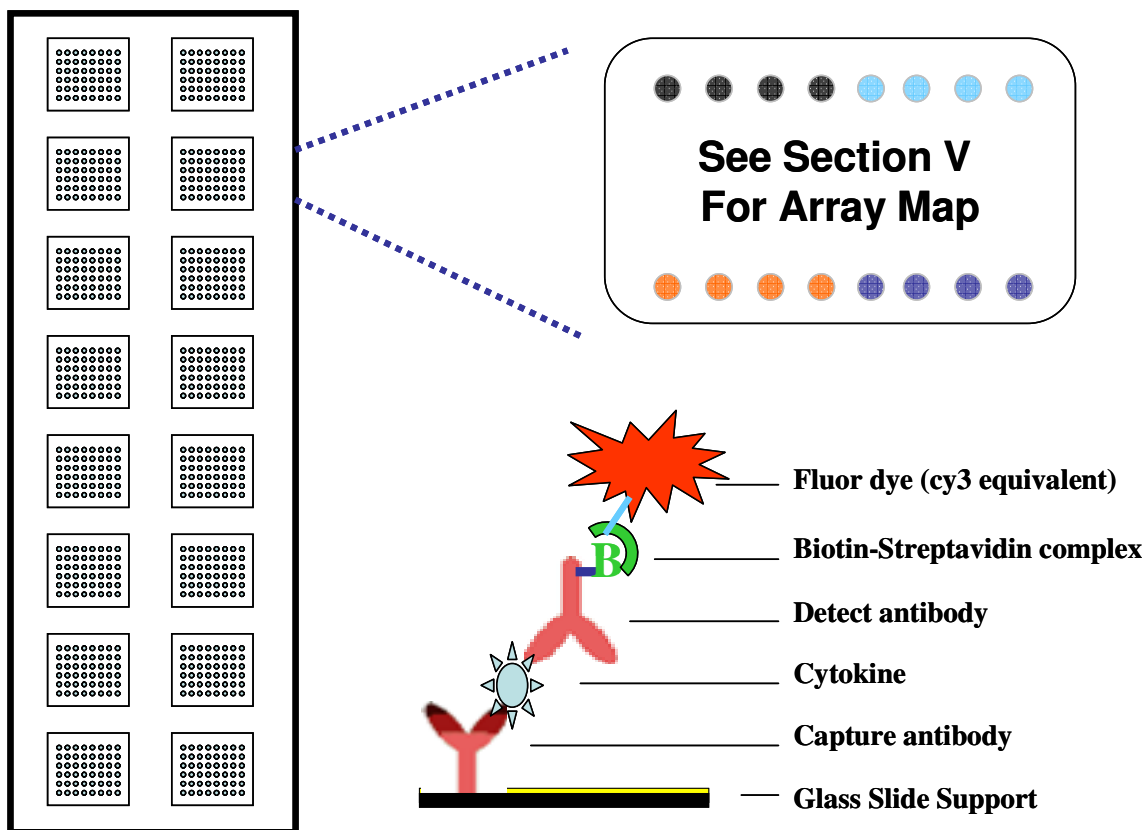
**RayBiotech, Inc.**

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

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Cytokine Detected (10)	CCL3L1, IFN $\alpha$ , IL-1 $\alpha$ , IL-1ra, IL-13, IL-17A, IL-18, MIG, MIP-1 $\beta$ , PECAM-1
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 $\mu$ l 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 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 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.

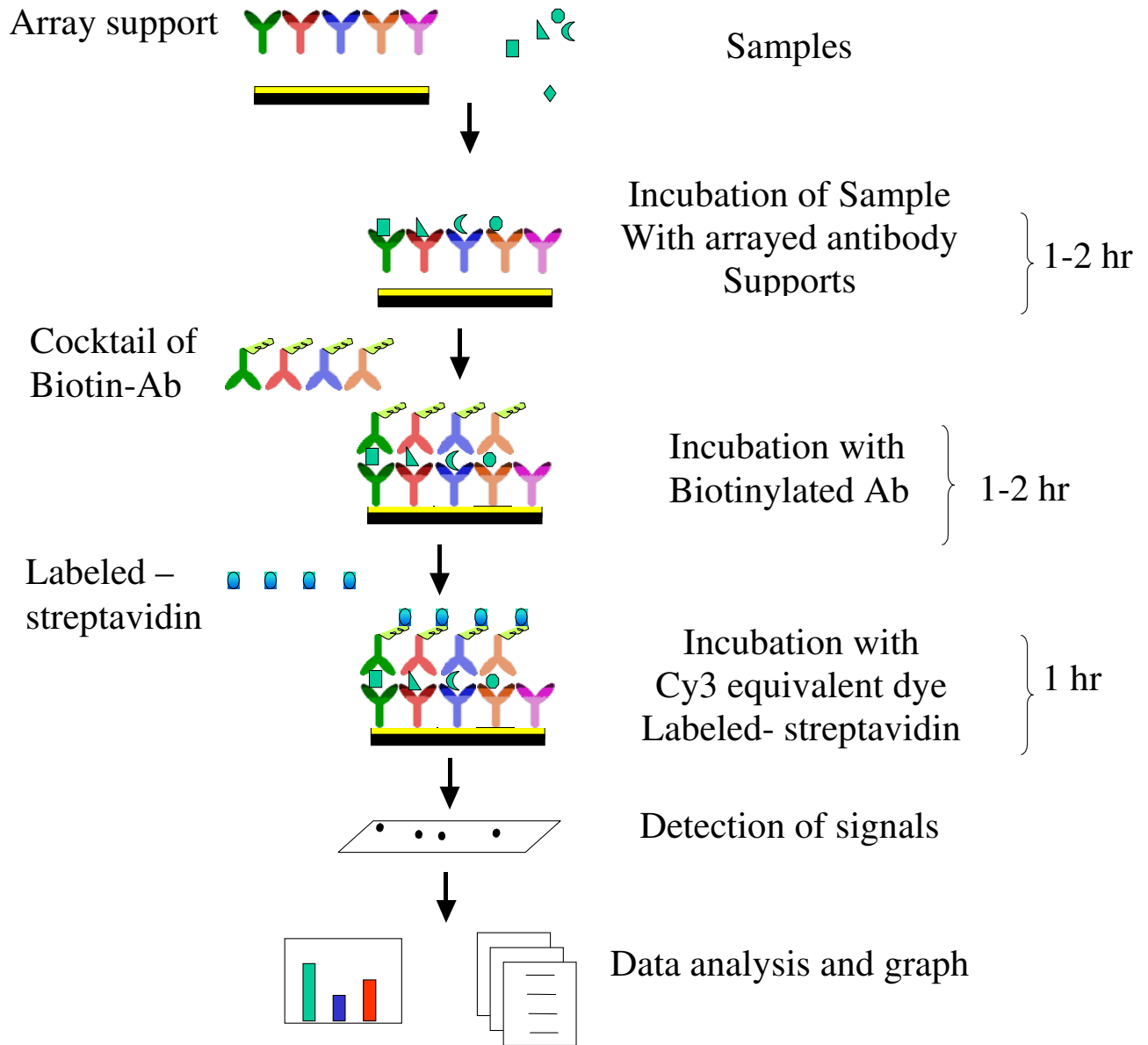
The Quantibody<sup>®</sup> array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of 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<sup>®</sup> 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 360 human or 160 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



## 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	1-Slide kit	2-Slide kit
1	Quantibody® Array Glass Slide	1	2
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
6	Detection antibody cocktail	1	2
7	Cy3 equivalent dye-conjugated Streptavidin	1	2
8	Slide Washer/Dryer	1	1
9	Adhesive device sealer	5	10
10	Manual	1	1

\* See Section VI for detailed cytokine concentrations after reconstitution.

### 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 contain 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 fluids, 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 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<sup>0</sup>C. Make sure to cover the incubation chamber tightly to prevent evaporation.



## IV. Protocol

### ***READ ENTIRE PROTOCOL BEFORE STARTING***

#### **A. Completely air dry the glass slide**

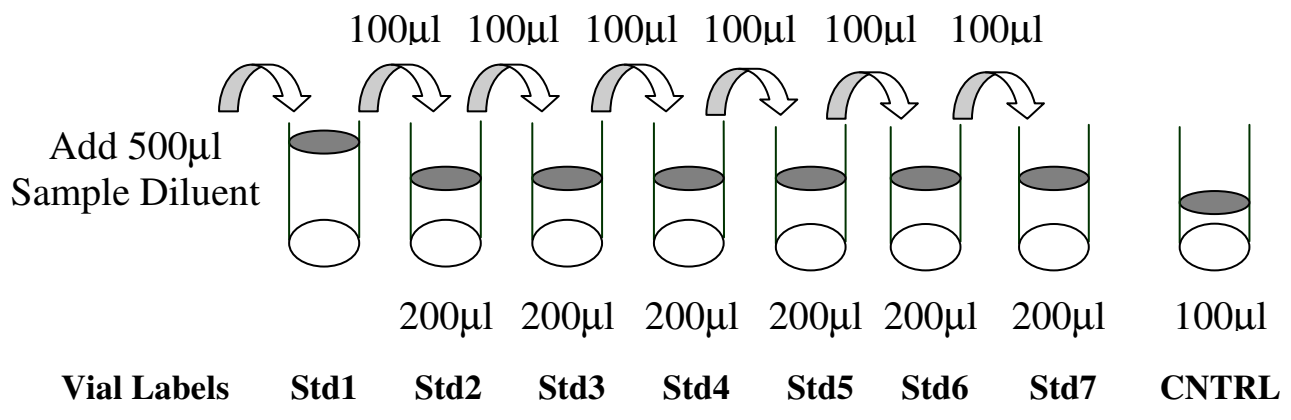
1. Take the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

*Note: Incomplete drying of slides before use may cause the formation of streaks or “comet tails” on 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  $-80^{\circ}\text{C}$  for future use.*

#### **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 gentle mixing. Label 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 of Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100ul of 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 are different, the serial concentrations from Std1 to Std7 for each cytokine are varied and 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. (Longer incubation time is preferable for higher signals)

*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<sup>0</sup>C for best results.*

## 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 ddH<sub>2</sub>O 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 entire 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. (Longer incubation time is preferable for higher signals and backgrounds)
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 after each wash step.

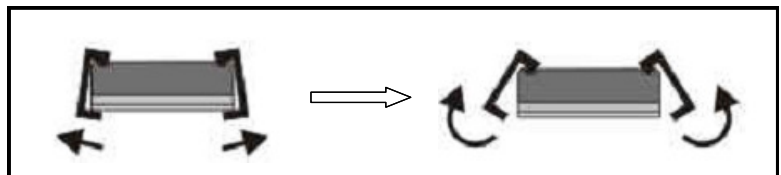
## **E. Incubation with Cy3 equivalent dye -Streptavidin and wash**

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
13. Add 80  $\mu$ 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  $\mu$ 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)*



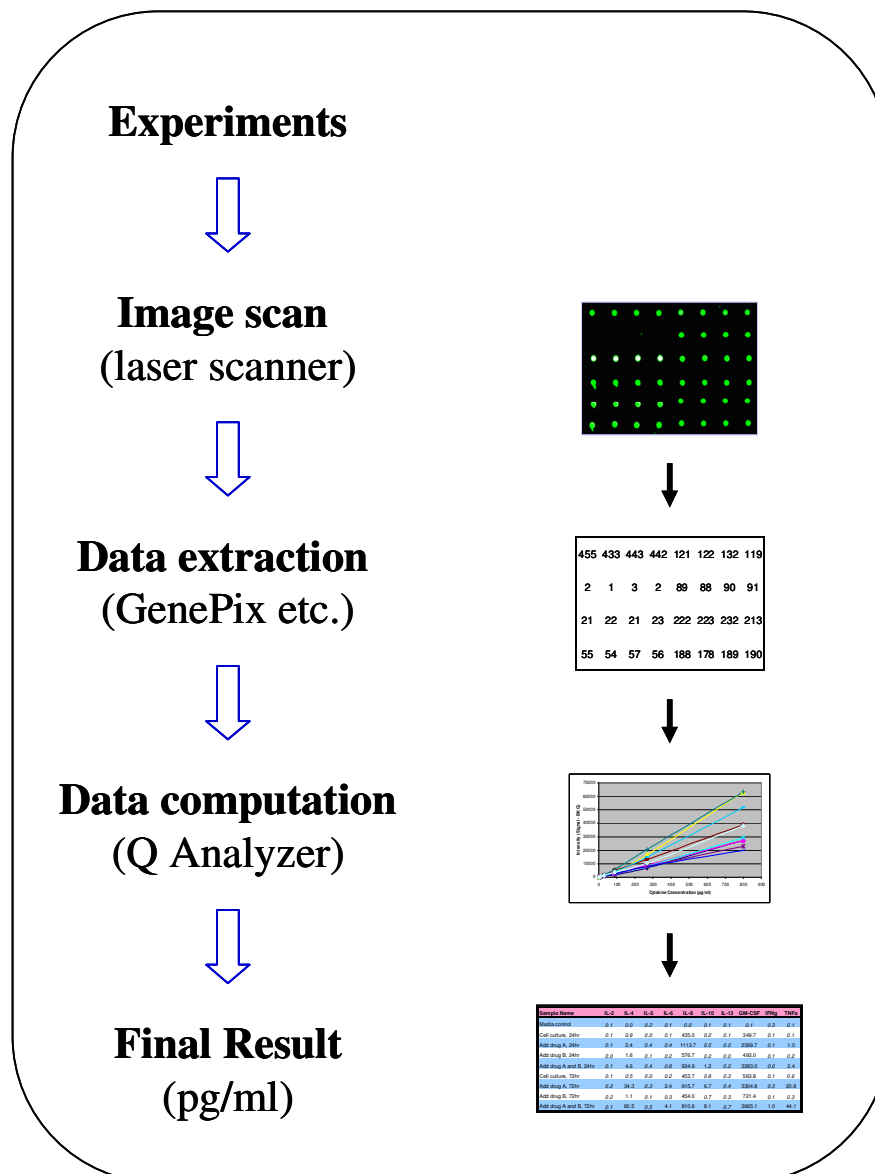
16. Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the entire slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) with gentle shaking at room 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 with a compressed N<sub>2</sub> stream.
  - Or gently apply suction with a pipette to remove buffer 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 vary 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

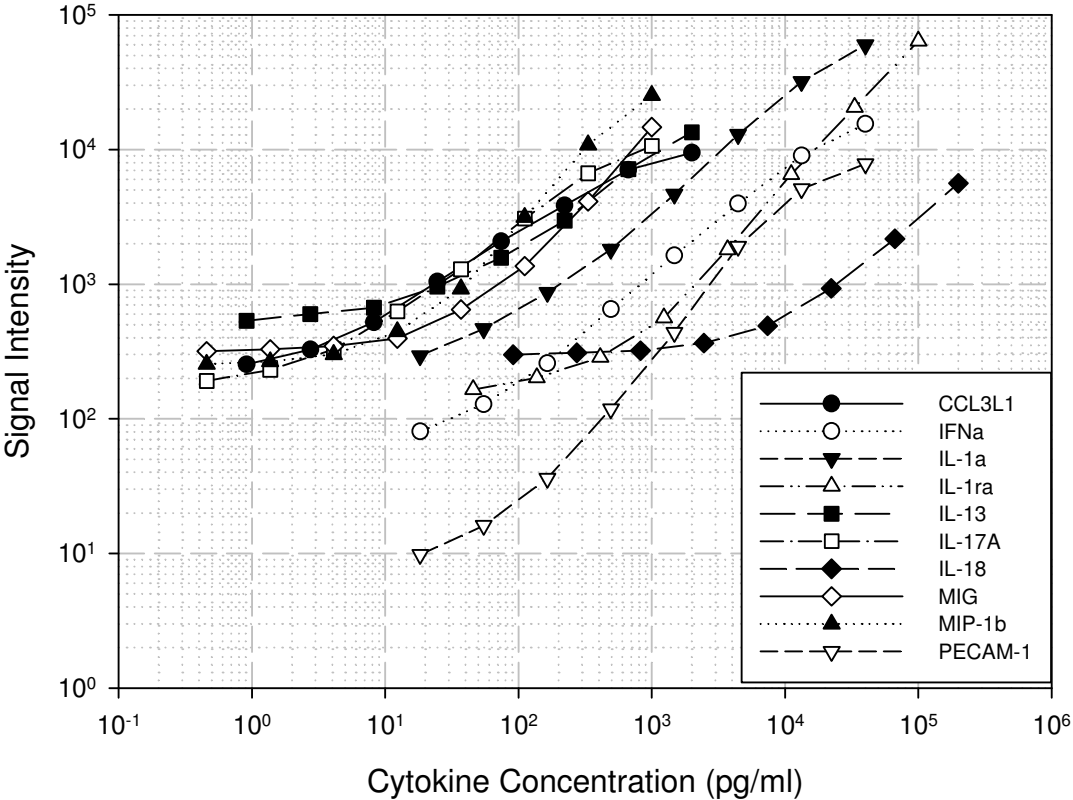
19. Data extraction can be done with most microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). For quantitative data analysis, our Quantibody® Q-Analyzer software is available. It gives visual output as well as digital values. More information can be found in section VIII.



# V. Cytokine Array Map & Standard Curves

<b>POS1</b>	<b>POS2</b>
<b>CCL3L1</b>	<b>IFN<math>\alpha</math></b>
<b>IL-1<math>\alpha</math></b>	<b>IL-1ra</b>
<b>IL-13</b>	<b>IL-17A</b>
<b>IL-18</b>	<b>MIG</b>
<b>MIP-1<math>\beta</math></b>	<b>PECAM-1</b>

## QAP-CYT-2 Standard Curves



## VI. 8-Point Standards

After reconstitution and serial dilution of the lyophilized cytokine standard mixes, the concentrations used for generating the 8-point cytokine standard curve of a given antigen are 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).

*Serial standard concentration (pg/ml)*

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
CCL3L1	0	3	8	25	74	222	667	2,000
IFN $\alpha$	0	55	165	494	1,481	4,444	13,333	40,000
IL-1 $\alpha$	0	55	165	494	1,481	4,444	13,333	40,000
IL-1ra	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-13	0	14	41	123	370	1,111	3,333	10,000
IL-17A	0	1	4	12	37	111	333	1,000
IL-18	0	274	823	2,469	7,407	22,222	66,667	200,000
MIG	0	1	4	12	37	111	333	1,000
MIP-1 $\beta$	0	1	4	12	37	111	333	1,000
PECAM-1	0	55	165	494	1,481	4,444	13,333	40,000

*Panel cross-reactivity*

CAB\DAB	CCL3L1	IFN $\alpha$	IL-1 $\alpha$	IL-1ra	IL-13	IL-17A	IL-18	MIG	MIP-1 $\beta$	PECAM-1
CCL3L1	9351	98	45	93	39	121	38	68	52	38
IFN $\alpha$	43	6275	41	69	42	35	40	67	58	42
IL-1 $\alpha$	33	27	9711	79	28	66	47	60	40	28
IL-1ra	13	87	16	13272	15	93	8	19	16	9
IL-13	38	89	45	106	8345	160	37	100	46	38
IL-17A	33	97	38	74	87	19732	55	59	41	33
IL-18	48	7	52	58	35	81	6578	64	61	42
MIG	44	119	44	81	60	219	40	10639	55	38
MIP-1 $\beta$	41	104	46	71	42	128	39	60	10935	40
PECAM-1	3	77	6	8	1	1	0	5	3	7171



## VII. System Recovery

The antibody pairs used in the kit have been tested to recognize their specific antigen. Analysis of samples containing only a single recombinant protein found no cross-reactivity with other proteins. The spiking recovery rate of the cytokines by the kit in 2x diluted porcine serum (SR), porcine plasma EDTA (PLE), porcine plasma citrate (PLC) and porcine plasma heparin (PLH) are listed in the following tables.

(pg/ml)	Spiking	SR	SR+Ag	SR%	PLE	PLE+Ag	PLE%
CCL3L1	500	98	482	77%	75	347	54%
IFN $\alpha$	10,000	447	6,393	59%	108	11,517	114%
IL-1 $\alpha$	50,000	196	33,773	67%	131	28,241	56%
IL-1ra	50,000	678	64,112	127%	3,445	45,700	85%
IL-13	250	62	184	49%	37	170	53%
IL-17A	250	14	248	94%	379	622	97%
IL-18	25,000	14,323	40,332	104%	7,569	29,912	89%
MIG	250	99	399	120%	212	416	82%
MIP-1 $\beta$	250	190	326	54%	134	322	75%
PECAM-1	20,000	61	12,663	63%	95	13,306	66%

(pg/ml)	Spiking	PLH	PLH+Ag	PLH%	PLC	PLC+Ag	PLC%
CCL3L1	500	87	334	50%	21	255	47%
IFN $\alpha$	10,000	25	12,731	127%	192	11,597	114%
IL-1 $\alpha$	50,000	188	29,261	58%	183	28,415	56%
IL-1ra	50,000	2,033	70,969	138%	1,967	65,838	128%
IL-13	250	13	122	43%	0	94	38%
IL-17A	250	29	261	93%	18	278	104%
IL-18	25,000	7,267	30,663	94%	18,350	38,699	81%
MIG	250	102	264	65%	76	357	112%
MIP-1 $\beta$	250	318	266	-	179	242	25%
PECAM-1	20,000	61	11,453	57%	50	12,412	62%

## 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 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.

## IX. Troubleshooting guide

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
<b>Weak Signal</b>	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 step to overnight
	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.
<b>Uneven signal</b>	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
<b>Poor standard curve</b>	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	"Comet tail" or streak formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard 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.
<b>High background</b>	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. 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 multiplex 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

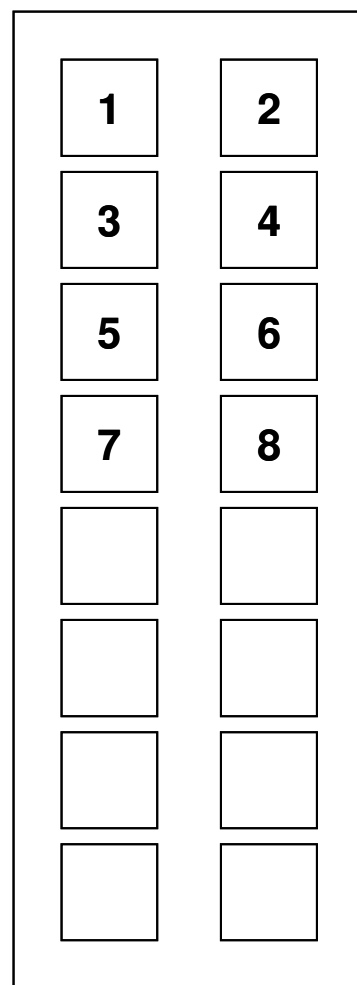
Date: \_\_\_\_\_

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		



## XII. How to Choose Quantibody® Products?

### Species-based selection:

- **Human:** (QAH-)
- **Mouse:** (QAM-)
- **Rat:** QAR-CYT-1, QAR-CYT-2, QAR-CYT-3, QAR-INF-1
- **Porcine:** QAP-CYT-1
- **Non-Human Primates (NHP):** QAN-CYT-1
- **Canine:** QAC-CYT-1

### Function-based selection:

- **TH1/TH2/TH17 Array:** QAH-TH-1, QAH-TH17, QAM-TH17
- **Inflammation Arrays:** QAH-INF-1, QAH-INF-2, QAH-INF-3; QAM-INF-1; QAR-INF-1
- **Angiogenesis Arrays:** QAH-ANG-1, QAH-ANG-2, QAH-ANG-3, QAH-ANG-1000
- **Chemokine Arrays:** QAH-CHE-1, QAM-CHE-1
- **MMP Array:** QAH-MMP-1
- **Immunoglobulin Isotype Array:** QAH-ISO-1; QAM-ISO-G1

### Cytokine Number-based selection:

- **320 cytokines:** QAH-CAA-7000
- **280 cytokines:** QAH-CAA-6000
- **240 cytokines:** QAH-CAA-5000
- **200 cytokines:** QAH-CAA-4000
- **160 cytokines:** QAH-CAA-3000, QAM-CAA-3000
- **120 cytokines:** QAH-CAA-2000; QAM-CAA-2000
- **80 cytokines:** QAH-CAA-1000; QAM-CAA-1000
- **60 cytokines:** QAH-ANG-1000; QAM-CYT-Q2000
- **40 cytokines:** QAH-INF-3, QAH-CHE-1, QAH-GF-1, QAH-REC-1, QAH-CYT-4, QAH-CYT-5, QAH-CYT-6, QAH-CYT-7, QAM-INF-1, QAM-CYT-4, QAM-CYT-5, QAM-CYT-6
- **30 cytokines:** QAH-ANG-2, QAH-ANG-3, QAM-INT-1000, QAR-CYT-3, QAM-CHE-1
- **20 cytokines:** QAH-CYT-1, QAH-CYT-2, QAM-CYT-1, QAM-CYT-2, QAM-CYT-3, QAM-INT-1, QAH-TH17-1, QAM-TH17-1
- **10 cytokines:** QAH-TH-1, QAH-INF-1, QAH-INF-2, QAH-ANG-1, QAH-MMP-1, QAH-ADI-1, QAM-INT-2, QAR-CYT-1, QAR-CYT-2, QAR-INF-1, QAN-CYT-1, QAP-CYT-1, QAH-IGF-1
- **less than 10 cytokines:** QAH-ISO-1, QAH-ADI-2, QAP-CYT-1, QAM-ISO-G1

### Purpose-based selection --- Custom Arrays

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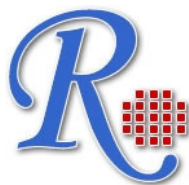
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