

Quantibody[®] Equine Cytokine Array 1

--Quantitative measurement of 10 Equine cytokines

Patent Pending Technology

User Manual (Version August 2013)

Cat # QAE-CYT-1



RayBiotech, Inc.

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

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

Cytokine Detected (10)	IFN γ , IL-1 α , IL-1ra, IL-2, IL-4, IL-8, IL-10, IL-15, MCP-1, VEGF
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	6 hrs

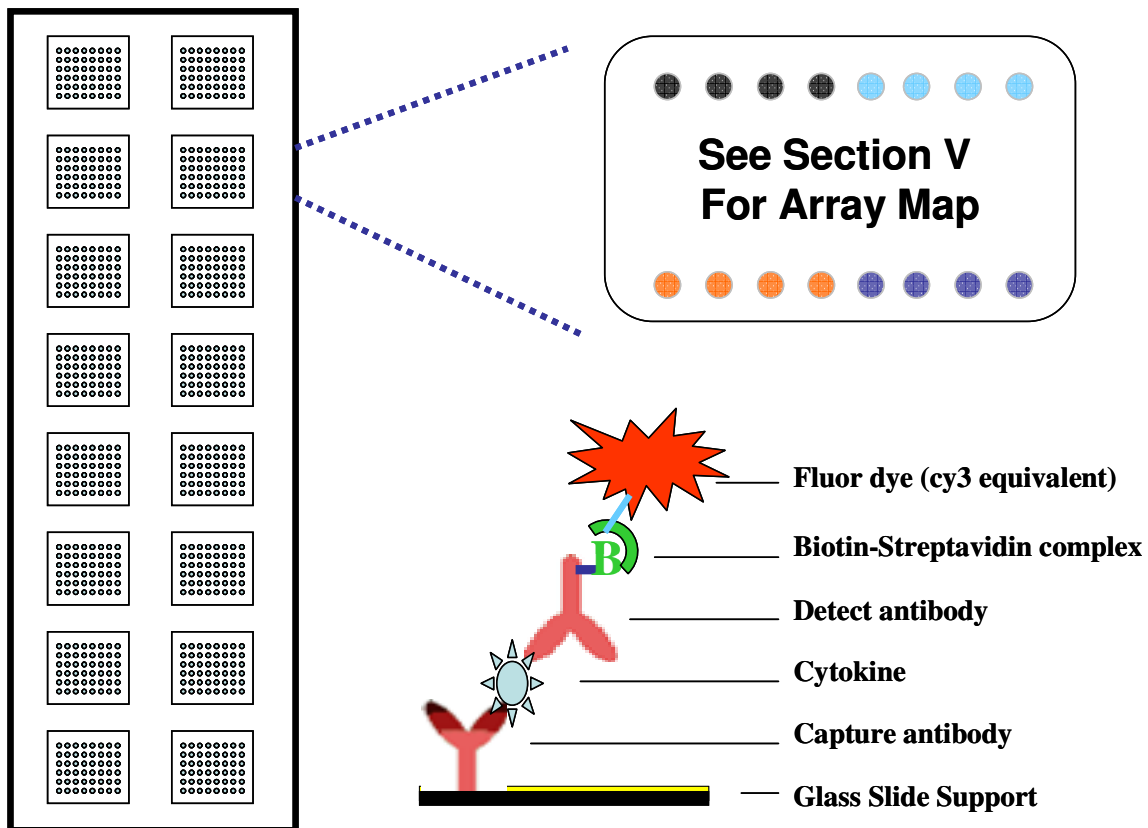


TABLE OF CONTENTS

I.	Overview.....	1
	Introduction.....	3
	How It Works	5
II.	Materials Provided.....	6
	Additional Materials Required.....	6
III.	General Considerations.....	7
	A. Preparation of Samples.....	7
	B. Handling Glass Slides.....	7
	C. Incubation.....	7
IV.	Protocol.....	8
	A. Complete Air Dry the Glass Slide.....	8
	B. Prepare Cytokine Standard Dilutions.....	8
	C. Blocking and Incubation.....	9
	D. Incubation with Detection Antibody Cocktail.....	10
	E. Incubation with Cy3 Equivalent Dye-Streptavidin....	11
	F. Fluorescence Detection.....	11
	G. Data Analysis.....	13
V.	Cytokine Array Map & Standard Curves.....	14
VI.	8-Point Standards.....	15
VII.	System Recovery.....	16
VIII.	Quantibody [®] Q-Analyzer	17
IX.	Troubleshooting Guide.....	18
X.	Select Quantibody [®] Publications.....	19
XI.	Experimental Record Form.....	20
XII.	How to Choose Quantibody [®] Products.....	21

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.

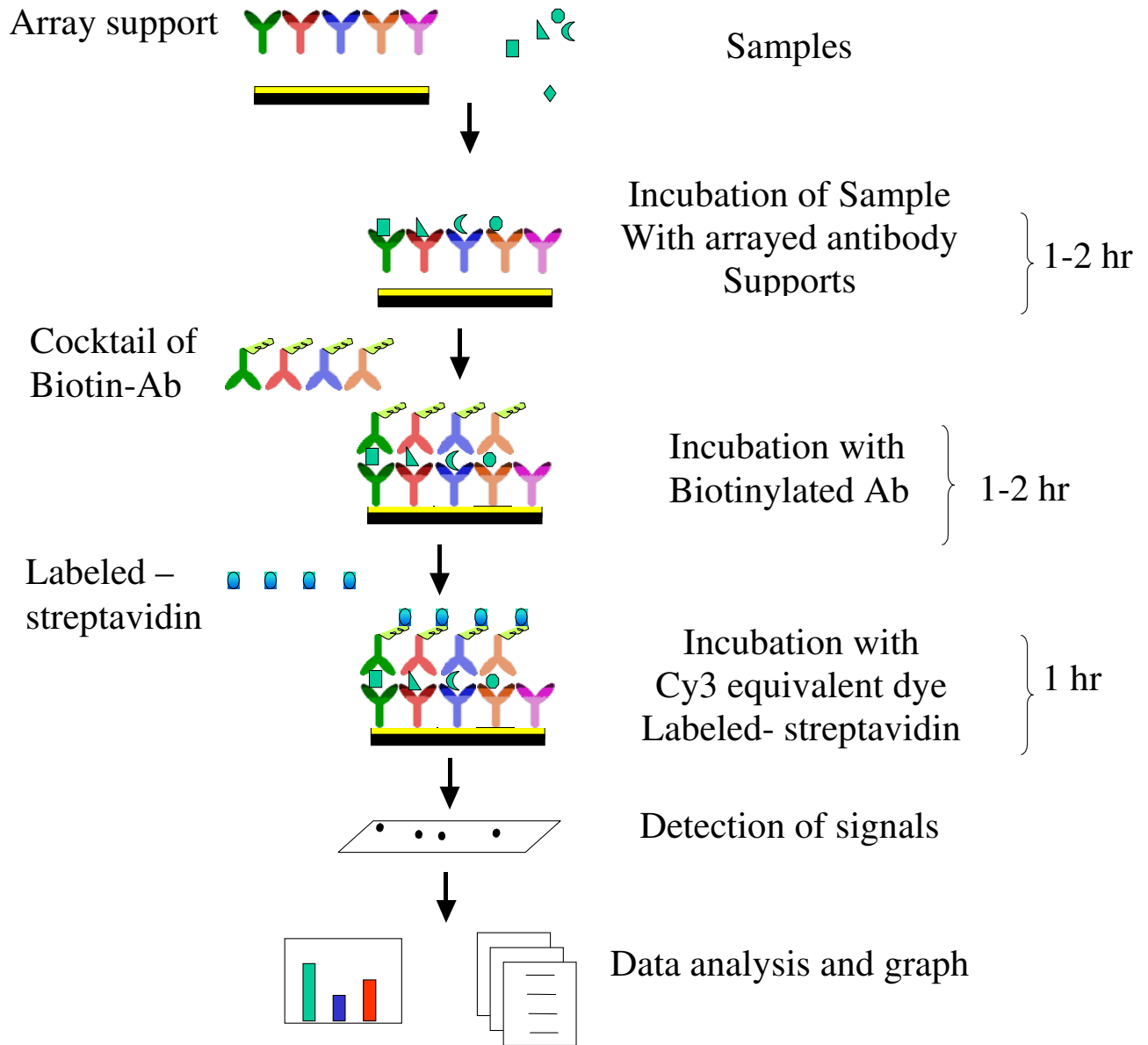
The Quantibody[®] array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and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

onto a glass support, quantitative, multiplex detection of cytokines in one experiment is made possible.

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

Quantibody[®] array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Our current high density Quantibody kits allow scientists to quantitatively determine the concentration of 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 contains cytokines.
- We recommend the following parameters for other samples:
50 to 100 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

B. Handling glass slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Handle glass slide in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, this will enable you to distinguish one slide from the other.

C. Incubation

- Completely cover the array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µl of sample or reagent is used.
- Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4⁰C. 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

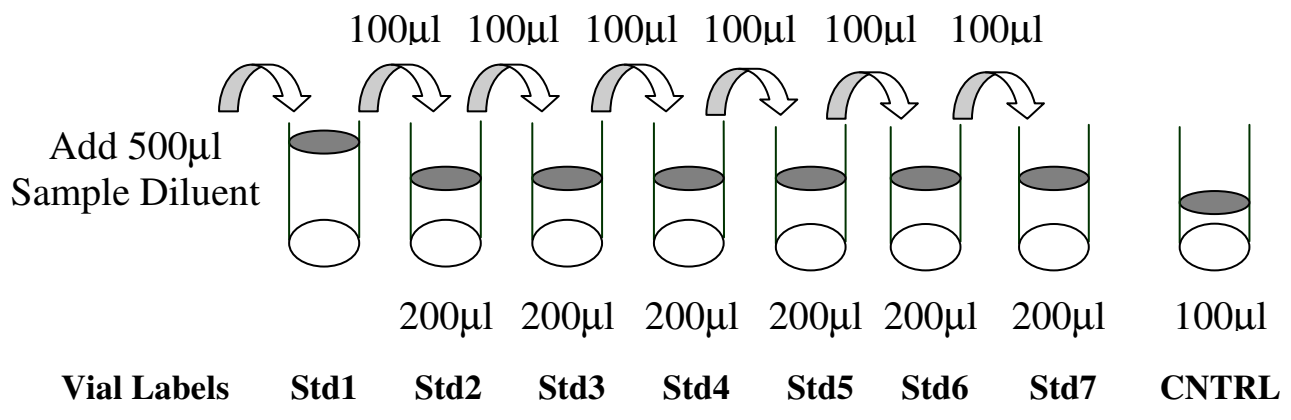
1. Take the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

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°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 4⁰C 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 ddH₂O to generate the required volume of 1x Wash Buffer I and 1x Wash Buffer II. For example 100 µl of 20x Wash Buffer I would be diluted to a final volume of 2,000 µl.
- Decant the samples from each well, and wash 5 times (5 min each) with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer after each wash step.
- (*Optional for Cell and Tissue Lysates*) Put the glass slide with frame into a box with 1x Wash Buffer I (cover the 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.

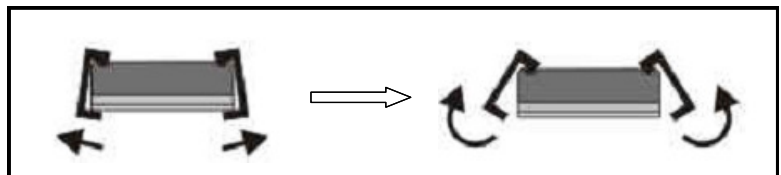
E. Incubation with Cy3 equivalent dye -Streptavidin and wash

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
13. Add 80 μ l of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in a dark room. Incubate at room temperature for 1 hour.
14. Decant the samples from each well, and wash 5 times with 150 μ l of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

F. Fluorescence Detection

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

(Be careful not to touch the surface of the array side)



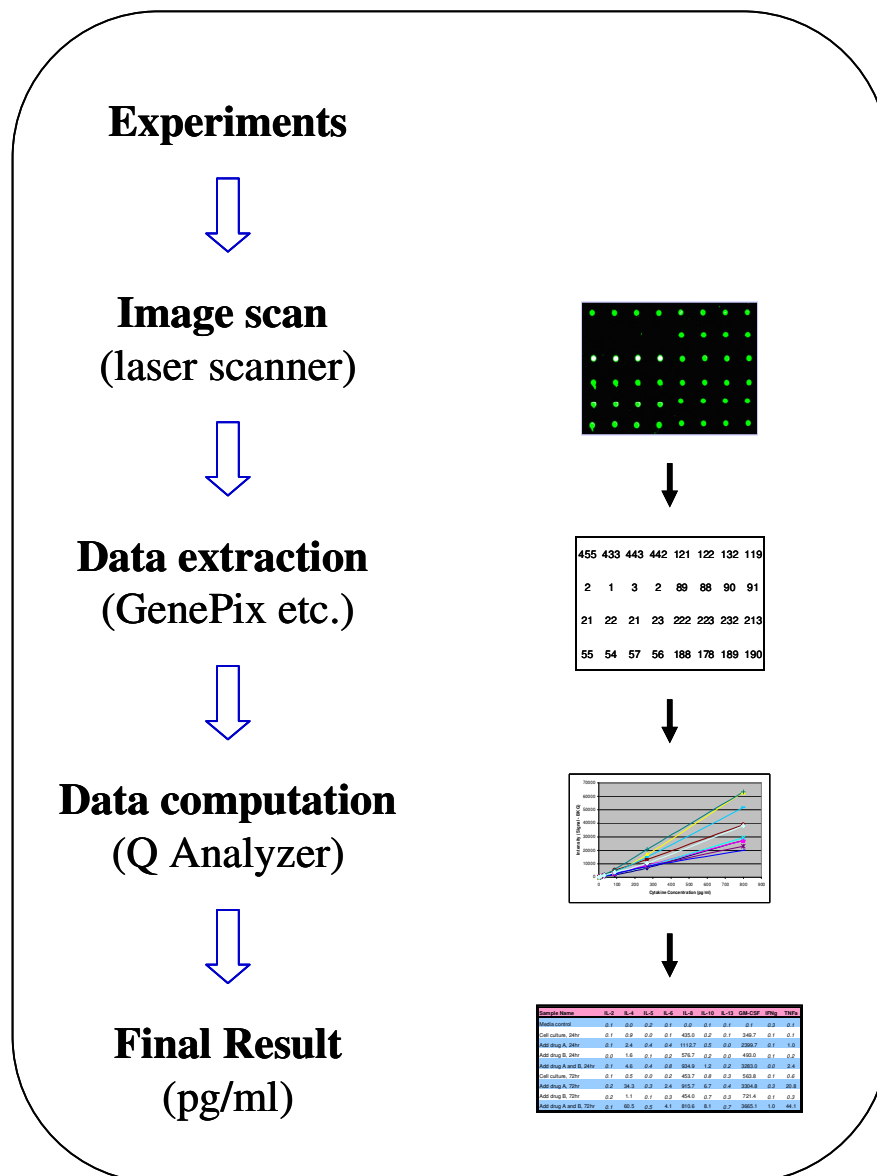
16. Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the entire slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) with gentle shaking at room temperature for 5 minutes then decant Wash Buffer II.
17. Remove buffer droplets completely by one of the following ways:
 - Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
 - Or, dry the glass slide by a compressed N₂ stream.
 - Or gently apply suction with a pipette to remove 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

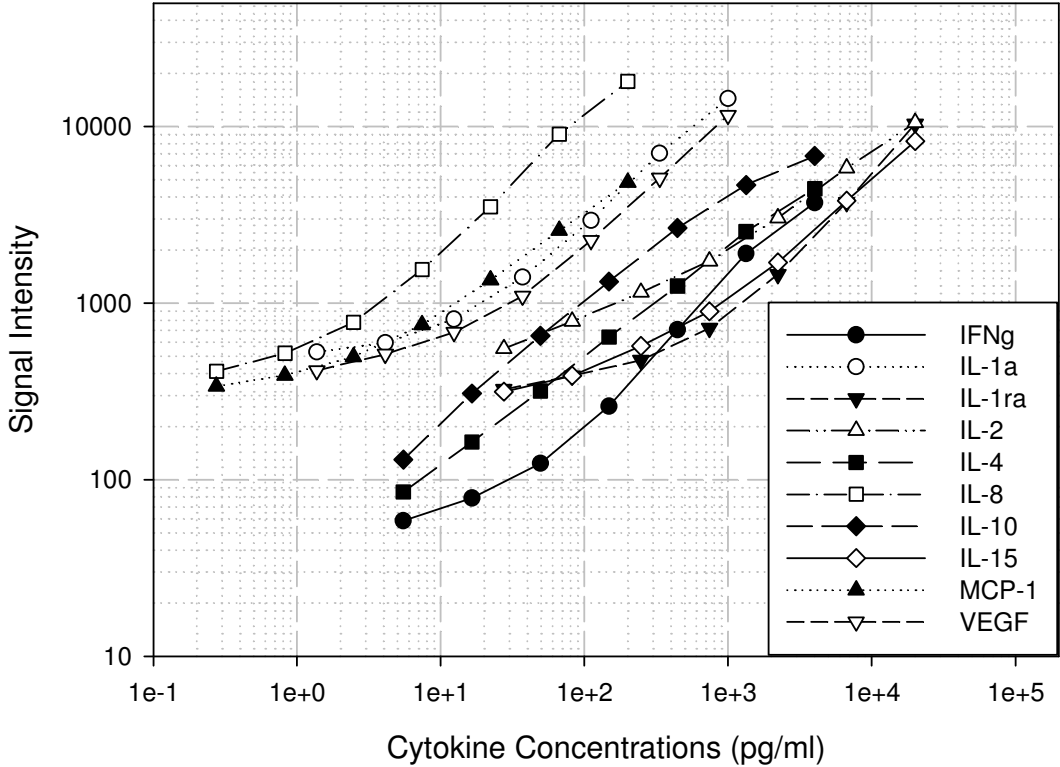
19. Data extraction can be done with most microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). For quantitative data analysis, our Quantibody[®] Q-Analyzer software is available. It gives visual output as well as digital values. More information can be found in section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



V. Cytokine Array Map & Standard Curves

POS1	POS2
IFNγ	IL-1α
IL-1ra	IL-2
IL-4	IL-8
IL-10	IL-15
MCP-1	VEGF

Equine Cytokine Array 1 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)	Control	Std7	Std6	Std5	Std4	Std3	Std2	Std1
IFN γ	0	5	16	49	148	444	1,333	4,000
IL-1 α	0	1	4	12	37	111	333	1,000
IL-1ra	0	27	82	247	741	2,222	6,667	20,000
IL-2	0	27	82	247	741	2,222	6,667	20,000
IL-4	0	5	16	49	148	444	1,333	4,000
IL-8	0	0	1	2	7	22	67	200
IL-10	0	5	16	49	148	444	1,333	4,000
IL-15	0	27	82	247	741	2,222	6,667	20,000
MCP-1	0	0	1	2	7	22	67	200
VEGF	0	1	4	12	37	111	333	1,000

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 negligible cross-reactivity with other proteins. The spiking recovery rates of the cytokines by the kit in 2x diluted Equine serum (SR), and plasma EDTA (PLE) are listed in the following tables.

Equine cytokine array 1 cross-reactivity test

	IFN γ	IL-1 α	IL-1ra	IL-2	IL-4	IL-8	IL-10	IL-15	MCP-1	VEGF
IFN γ	3133	5	12	10	8	8	12	9	2	9
IL-1 α	62	5508	86	72	75	45	183	40	55	53
IL-1ra	27	16	8880	28	20	19	51	45	14	14
IL-2	23	14	43	2438	24	16	82	24	21	20
IL-4	10	6	9	11	2480	2	12	6	2	41
IL-8	67	53	66	62	39	7907	155	24	31	31
IL-10	0	2	7	11	7	1	4491	7	3	8
IL-15	31	23	28	34	37	27	74	3467	21	20
MCP-1	37	31	59	32	37	27	115	20	5531	26
VEGF	73	40	77	83	57	31	142	31	32	3079

The spiking recovery rate for Equine serum and plasma

(pg/ml)	Spiking	SR	SR+Ag	SR%	PLE	PLE+Ag	PLE%
IFN γ	2,000	35	2,079	102%	0	803	40%
IL-1 α	200	0	126	63%	0	74	37%
IL-1ra	10,000	818	17,683	169%	99	5,295	52%
IL-2	4,000	0	3,360	84%	0	3,338	83%
IL-4	800	2	632	79%	0	494	62%
IL-8	40	123	159	91%	16	49	84%
IL-10	400	4	397	98%	1	217	54%
IL-15	2,000	1	1,327	66%	0	1,243	62%
MCP-1	40	84	128	109%	8	23	39%
VEGF-A	100	0	84	84%	0	66	66%

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

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time or change sample incubation 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.
Uneven signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard at room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated
	Use freeze-thawed cytokine standards	Always use a new cytokine standard vial for a new experiment. Discard any leftovers
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from drying out during experiment

X. 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		

1	2
3	4
5	6
7	8

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
- **Non-Human Primates (NHP):** QAN-CYT-1
- **Porcine:** QAP-CYT-1
- **Canine:** QAC-CYT-1
- **Equine:** QAE-CYT-1
- **Feline:** QAF-CYT-1

Function-based selection:

- **TH1/TH2/TH17 Array:** QAH-TH-1, QAH-TH17, QAM-TH17
- **IL-1 Family Cytokine and Receptor Arrays:** QAH-IL1F-1, QAH-IL1R-1
- **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; AAM-ISO-G1
- **Dried Eye Disease Array:** QAH-DED-1
- **Periodontal Disease Array:** QAH-PDD-1
- **Bone Metabolism Array:** QAH-BMA-1, QAH-BMA-2, QAH-BMA-1000
- **Obesity Arrays:** QAH-ADI-1, QAH-ADI-2, QAH-ADI-3
- **Adhesion Molecule Arrays:** QAH-CAM-1

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,
- **30 cytokines:** QAH-ANG-2, QAH-ANG-3, QAR-CYT-3, QAM-CHE-1
- **20 cytokines:** QAM-INT-1, QAH-TH17-1, QAM-TH17-1
- **10 cytokines:** QAH-TH-1, QAM-INT-2, QAR-INF-1, QAN-CYT-1, QAP-CYT-1,
- **less than 10 cytokines:** QAH-ISO-1, QAH-ADI-2, AAM-ISO-G1

Purpose-based selection --- Custom Arrays

- Choose from over 800 cytokine pool; Any kind; Any number
- Order slide only or full service in house
- Desired marker not in our pool? No problem! For certain developmental fee, we may be able to add the marker to your panel if the paired antibodies are available on the market.

Check our website regularly for updated Quantibody® products

Note:

Quantibody[®] is the trademark of RayBiotech, Inc.

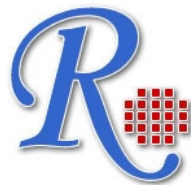
Cytokine protein arrays are RayBiotech patent-pending technology.

This product is intended for research only and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

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