# **Quantibody<sup>®</sup> Human Cytokine Array 27**

Quantitative measurement of 40 human cytokines

Catalog #: QAH-CYT-27

User Manual Last revised July 18, 2019

Caution: Extraordinarily useful information enclosed



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**

Section	n	Page #
I.	Overview	3
II.	Introduction	3
III.	How It Works	5
IV.	Materials Provided	6
V.	Storage	6
VI.	Additional Materials Required	6
VII.	General Considerations A. Sample Preparation B. Handling Glass Slides C. Incubation	7 7 7 7
VIII.	Protocol A. Completely Air Dry The Glass Slide B. Prepare Cytokine Standard Dilutions C. Blocking & Incubation D. Incubation with Biotinylated Antibody Cocktail & Wash E. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash F. Fluorescence Detection G. Data Analysis	8 8 9 10 10 11 12
IX.	Array Map & Standard Curves	13
X.	Standard Concentrations	14
XI.	Spiking & Recovery	15
XII.	Q-Analyzer: Data Analysis Software	16
XIII.	Troubleshooting Guide	17
XIV.	Select Publications	18
XV.	Experiment Record Form	19
XVI.	How To Choose A Quantibody®	20

Please read the entire manual carefully before starting your experiment

# I. Overview

Cytokines Detected (40)	15-PGDH, ALPL, ANGPTL2, Annexin A1, Attractin, Catalase, Cathepsin C, CD47, CD79A, CHIT1, CLUL1, CNDP1, CNP, COCO, ELF3, FABP3, FABP5, Fibromodulin, FLRT3, FOLR3, GFR alpha-3, GSTP1, IDS, IkB? alpha, JNK2, KLK6, KLK8, NELL1, NTH1, OBCAM, PRX1, SCCA1, Serpin A12, Sirtuin 3, SOD1, SOX10, SULT1B1, SULT1C2, SULT2B1, TIGIT  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

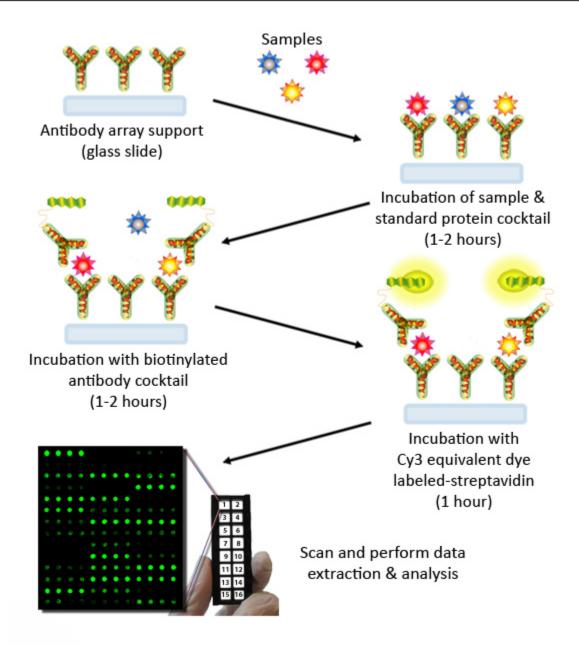
reviewed publications, including top-tier journals such as Cell and Nature.

The Quantibody<sup>®</sup> 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<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 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**



# IV. Materials Provided

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	QAH-CYT-27 S	Human Cytokine Array 27 Glass Slide	1	2
2	QA-SDB	Quantibody <sup>®</sup> 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-27 -STD	Human Cytokine Array 27 Lyophilized Standard Mix**	1 Vial	
6	QAH-CYT-27 B	Human Cytokine Array 27 Biotinylated Antibody Cocktail	1-25 µl	2 x 1-25 μl
7	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 μΙ	2 x 5 µl
8	QA-SWD	/D Slide Washer/Dryer		nl Tube
9	QA-ADH	Adhesive Film	1	2

<sup>\* 4</sup> slide kits are comprised of 2 separate 2 slide kits.

# 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

<sup>\*\*</sup> See Section X for detailed cytokine concentrations after reconstitution.

# 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<sup>®</sup> 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 dyestreptavidin incubation) may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

# VIII. Protocol

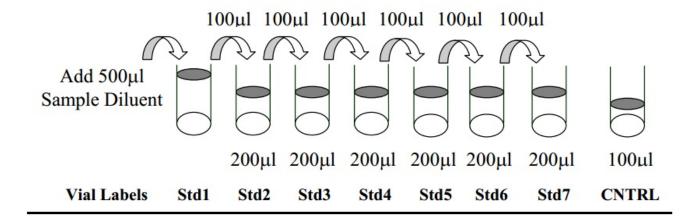
# A. Completely Air Dry The Glass Slide

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.



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  $\mu$ l of original or diluted serum, plasma, conditioned media, or other body fluid, or 250  $\mu$ g/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  $\mu$ l 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 µI 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

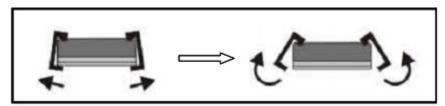
11. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I and then 2 times with 150 µl of 1x Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

# 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.
  - Decant the samples from each well, and wash 5 times (5 mins each) with 150
- 14. 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.

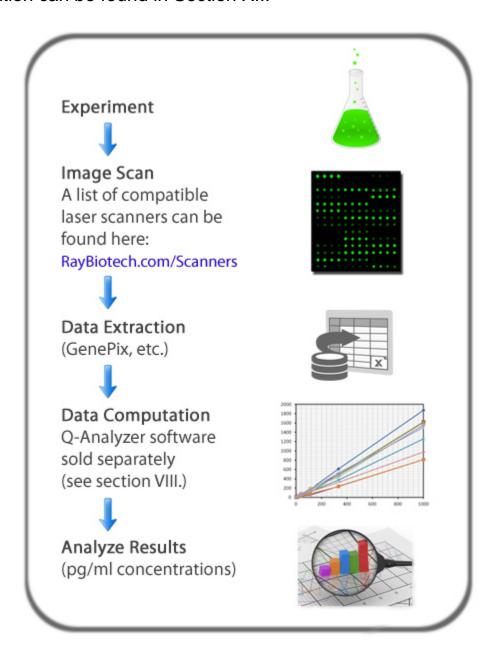
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains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

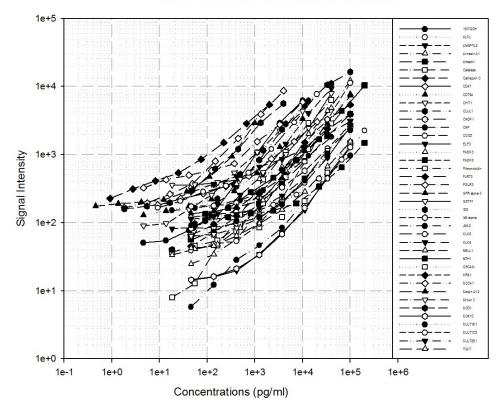
Need help analyzing all that data? Copy and paste your data into the Q-Analyzer Tool specific for this array, catalog number: **QAH-CYT-27-SW**. More information can be found in Section XII.



# IX. Array Map & Standard Curves

	(hCYT27 Map) Each antibody is printed in quadruplicate horizontally											
	1	2	3	4	1	2	3	4	1	2	3	4
Α		PO	S1			PC	S2			15-P	GDH	
В		AL	PL			ANG	PTL2			Anne	xin A1	
С		Attra	actin			Cata	lase			Cathe	psin C	
D	CD47				CD	79A		CHIT1				
E	CLUL1			CNDP1		CNP						
F	coco			ELF3			FABP3					
G	FABP5			Fibromodulin			FLRT3					
Н	FOLR3			GFR alpha-3			GSTP1					
I	IDS				IkB- alpha			JNK2				
J	KLK6			KLK8			NELL1					
K	NTH1			OBCAM		PRX1						
L	SCCA1				Serpin A12			Sirtu	uin 3			
M	SOD1				SO	SOX10 SULT1B1						
N		SUL	Γ1C2			SUL	Г2В1			TIC	GIT	

### **QAH-CYT-27 Standard Curves**



# X. Standard Concentrations

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

(pg/mll)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	St
15-PGDH	0	14	41	123	370	1,111	3,333	10,
ALPL	0	274	823	2,469	7,407	22,222	66,667	200
ANGPTL2	0	55	165	494	1,481	4,444	13,333	40.
Annexin A1	0	137	412	1,235	3,704	11,111	33.333	100
Attractin	0	137	412	1,235	3.704	11.111	33,333	100
Catalase	0	55	165	494	1,481	4,444	13,333	40,0
Cathepsin C	0	137	412	1,235	3,704	11,111	33,333	100
CD47	0	27	82	247	741	2,222	6,667	20,0
CD79A	0	14	41	123	370	1,111	3,333	10,0
CHIT1	0	14	41	123	370	1,111	3.333	10,0
CLUL1	0	137	412	1,235	3,704	11,111	33.333	100
CNDP1	0	137	412	1,235	3,704	11,111	33,333	100
CNP	0	55	165	494	1,481	4,444	13,333	40,0
COCO	0	137	412	1,235	3,704	11,111	33.333	100
ELF3	0	137	412	1,235	3,704	11,111	33,333	100
FAB P3	0	137	412	1.235	3,704	11,111	33.333	100
FAB P5	0	274	823	2,469	7,407	22,222	66,667	200
Fibromodulin	0	55	165	494	1,481	4,444	13.333	40,0
FLRT3	0	3	8	25	74	222	667	2,0
FOLR3	0	5	16	49	148	444	1,333	4.0
GFR alpha-3	0	1	4	12	37	111	3 3 3	1.0
GSTP1	0	55	165	494	1,481	4,444	13,333	40,0
IDS	0	55	165	494	1,481	4,444	13,333	40.0
lkB- alpha	0	137	412	1,235	3.704	11,111	33,333	100
JNK2	0	137	412	1,235	3,704	11,111	33,333	100
KLK6	0	27	82	247	741	2,222	6,667	20,0
KLK8	0	137	412	1,235	3,704	11,111	33,333	100
NELL1	0	55	165	494	1,481	4,444	13,333	40,0
NTH1	0	274	823	2,469	7,407	22,222	66,667	200
OBCAM	0	137	412	1 235	3,704	11,111	33.333	100
PRX1	0	137	412	1,235	3,704	11,111	33,333	100
SCCA1	0	14	41	123	370	1,111	3,333	10.
Serpin A12	0	137	412	1,235	3,704	11,111	33,333	100
Sirtuin 3	0	137	412	1,235	3,704	11,111	33,333	100
SOD1	0	5	16	49	148	444	1,333	4,0
SOX10	0	137	412	1,235	3,704	11,111	33,333	100
SULT1B1	0	137	412	1,235	3,704	11,111	33,333	100
SULT1C2	0	137	412	1,235	3,704	11,111	33,333	100
SULT2B1	0	137	412	1,235	3,704	11,111	33,333	100
TIGIT	1	137	412	1,235	3,704	11,111	33,333	100

# 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.

(pg/ml)	Spike-in	Serum	Serum+Ag	Serum%	CM	CM+Ag	CN
15-PGDH	5,000	0	4,721	94%	17	5,470	109
ALPL	100,000	0	55,227	55%	564	102,788	102
ANGPTL2	20,000	1,243	11,189	50%	0	24,363	122
Annexin A1	50,000	0	53,083	106%	0	82,115	124
Attractin	50,000	19,534	45,902	53%	29	66,229	132
Catalase	20,000	6,930	20,589	68%	0	22,674	113
Cathepsin C	50,000	0	31,827	64%	0	84,227	128
CD47	10,000	134	6,040	59%	371	10,147	98
CD79A	5,000	35	1,725	34%	138	7,941	156
CHIT1	5,000	1,248	3,738	50%	0	3.862	77
CLUL1	50,000	240	54,655	109%	6	56,852	114
CNDP1	50,000	394	32,691	65%	1,749	85,841	128
CNP	20,000	0	9,619	48%	707	22,867	111
coco	50.000	0	20,516	41%	0	69.343	139
ELF3	50,000	0	50,716	101%	999	72,017	142
FAB P3	50,000	901	47,837	94%	1.309	78,639	155
FAB P5	100,000	323	45,202	45%	2,934	141,192	138
Fibromodulin	20,000	0	11,278	58%	64	32,408	162
FLRT3	1,000	24	415	39%	6	1,562	156
FOLR3	2.000	707	2,211	75%	13	2.250	112
GFR alpha-3	500	8	597	118%	84	970	177
GSTP1	20,000	463	24,046	118%	1,445	32,461	155
IDS	20,000	1,211	16,624	77%	40	21,815	109
lkB- alpha	50,000	0	35,543	71%	819	80,812	120
JNK2	50,000	39	16,032	32%	2,192	75,587	147
KLK8	10,000	0	3,085	31%	186	9,338	92
KLK8	50,000	138	29,732	59%	292	41,730	83
NELL1	20,000	0	15,780	79%	24	30,393	152
NTH1	100,000	2,378	96,003	94%	3,547	87,675	84
OBCAM	50.000	0	27,915	56%	6,238	67,718	123
PRX1	50,000	50	38,976	78%	0	61,842	124
S C C A 1	5,000	131	3,972	77%	201	5,957	115
Serpin A12	50,000	596	20,103	39%	3,976	57,248	107
Sirtuin 3	50,000	0	24,103	48%	206	78,824	157
SOD1	2,000	318	1,837	76%	20	2,828	140
SOX10	50,000	0	33,852	68%	1,438	94,880	186
SULT1B1	50,000	699	56,045	111%	4,139	71,442	135
SULT1C2	50,000	200	65,536	131%	1,820	72,439	142
SULT2B1	50,000	75	45,717	91%	3,625	89,476	172
TIGIT	50,000	0	18,241	36%	340	78,787	157

# XII. Quantibody<sup>®</sup> 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-27-SW**.

# **Key features:**

- <u>Simplicity:</u> 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.
- <u>Two Positive Controls:</u> The program utilizes the two positive controls in each array for normalization.
- <u>Two Analytical Algorithms:</u> Users can choose either linear regression or log-log algorithms to meet their analytical needs.
- Two Data Outputs: standard curves and digital concentration.
- <u>User Intervention:</u> The program allows for user manual handling of outliers and other analytical data.
- <u>Lower and Upper Limits Determination</u>: The program automatically marks out the values below or above the detection range.
- <u>Standard Deviation:</u> 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		
	Inadequate detection	Increase laser power and PMT parameters		
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation		
Weak Signal	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.		
	Bubble formed during incubation	Decrease amount of rocking during incubations. check for bubble formation and remove bubbles.		
Uneven signal	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		
	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		
Poor standard curve	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.		
Curve	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.		
	Overexposure	Lower the PMT or signal gain.		
Liah	Dark spots	Completely remove wash buffer in each wash step.		
High background	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

 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

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

 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:
_aser 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
9	10
11	12
13	14
15	16

# XVI. How to Choose a Quantibody<sup>®</sup> 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<sup>®</sup> platform to detect 1000 human, 200 mouse, or 67 rat proteins. GLP-Compliant testing services are also available.

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

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



©2019 RayBiotech, Inc