

G-Series Human Cytokine Antibody Array 1200

A combination of 30 non-overlapping arrays to measure the relative
expression levels of 1200 human cytokines

Catalog #: GSH-CAA-1200

User Manual
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Caution:
Extraordinarily useful information enclosed



ISO 13485 Certified

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (1200)	Arrays Included: GSH-INF-3 (40); GSH-GF-1 (40); GSH-CHE-1 (40); GSH-REC-1 (40); GSH-CYT-4 (40); GSH-CYT-5 (40); GSH-CYT-6 (40); GSH-CYT-7 (40); GSH-CYT-8 (40); GSH-CYT-9 (40); GSH-CYT-10 (40); GSH-CYT-11 (40); GSH-CYT-12 (40); GSH-CYT-13 (40); GSH-CYT-14 (40); GSH-CYT-15 (40); GSH-CYT-16 (40); GSH-CYT-17 (40); GSH-CYT-18 (40); GSH-CYT-19 (40); GSH-CYT-20 (40); GSH-CYT-21 (40); GSH-CYT-22 (40); GSH-CYT-23 (40); GSH-CYT-24 (40); GSH-CYT-25 (40); GSH-CYT-26 (40); GSH-CYT-27 (40); GSH-CYT-28 (40); GSH-SAP-4 (40) <i>See Section IX for Array Map</i>
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

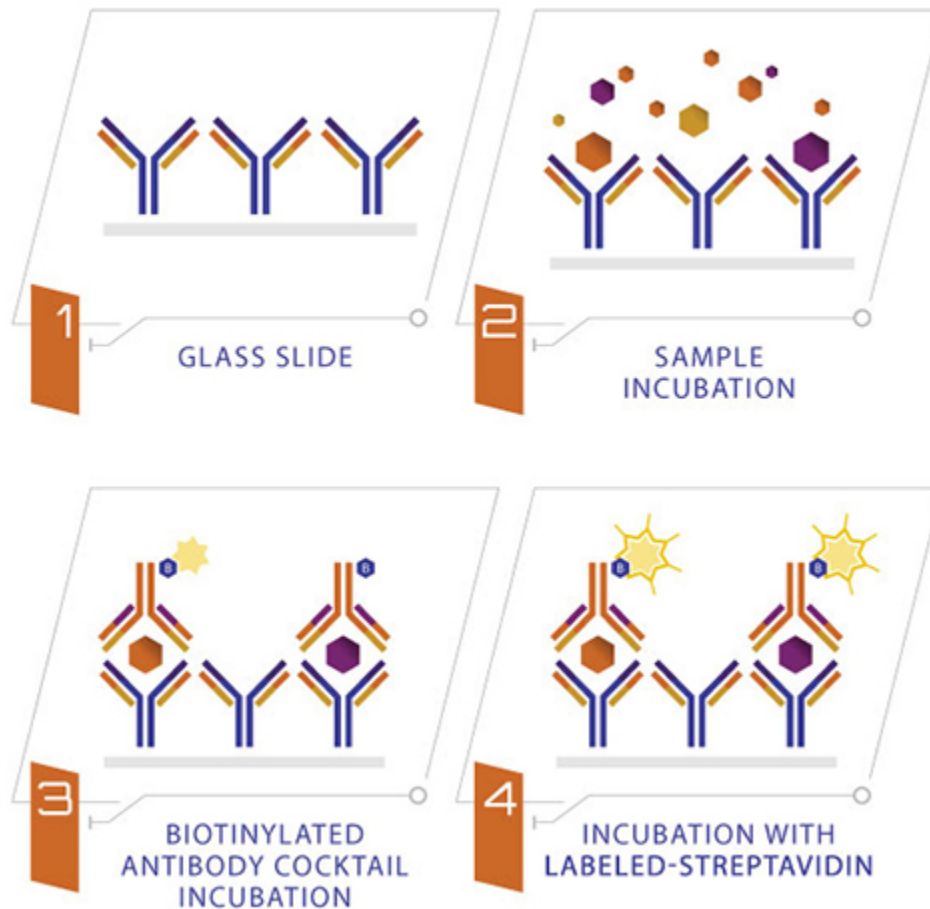
II. Introduction

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. RayBio® G-Series Arrays are glass slide-based antibody arrays which allow researchers to conduct rapid, accurate expression profiling of hundreds of cytokines, chemokines, growth factors, proteases, soluble receptors and other proteins from any biological fluid. Like a traditional sandwich-based ELISA, this array uses a matched pair of cytokine-specific antibodies for detection. After incubation with the sample, the target cytokines are captured by the antibodies printed on the solid surface. A second biotin-labeled

detection antibody is then added, which recognizes 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. Like the Quantibody[®] arrays, G-Series utilizes a highly sensitive and stable fluorescent readout which can be detected by most laser fluorescent scanner systems. After capturing the spot densities with a laser scanner, normalization of the raw data can be easily calculated by the researcher, or by a quick copy-paste into our excel-based Analysis Tool software.

This array as well as all catalog numbers beginning with 'GS' differ from the classic G-Series Arrays in a few important ways. First, each capture antibody is printed in quadruplicate instead of duplicate, delivering higher precision. Secondly, this array features the same antibody panels used in our Quantibody Arrays, allowing a seamless transition to our quantitative multiplex assay platform. Lastly, all 16 wells are spotted as sub-arrays, delivering easy handling of 16 samples simultaneously while consuming low sample volumes (10 - 100 µl per array).

III. How It Works



IV. Materials Provided

This product is a combination of multiple arrays. Items 1, 5, & 6 are array-specific.

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	[Array-Cat-#]S	Array-specific Glass Slide	1	2
2	QA-SDB	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	[Array-Cat-#]B	Array-specific Biotinylated Antibody Cocktail	1-25 μ l	2 x 1-25 μ l
6	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 μ l	2 x 5 μ l
7	QA-SWD	Slide Washer/Dryer	1 x 30 ml Tube	
8	QA-ADH	Adhesive Film	1	2

* 4 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, 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 μ l of total sample volume. To avoid matrix effects, we recommend using a minimum of 2x dilution for serum, plasma, cell culture media, or other body fluids, or 500 μ g/ml-1 mg/ml (after a 5-fold to 10-fold dilution to minimize the effects of any detergent(s)) total protein for cell and tissue lysates. Please be aware, more sample volume is required for combination arrays. For example, the minimum sample volume for a 10-array kit is 500 μ l, or 500 μ g cell lysate.

If you experience high background or if the fluorescent signal intensities 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 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 μ l 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

Note: *This product contains sets of reagents for different arrays. Always ensure you are using the proper glass slide and biotinylated antibody cocktail for the correct corresponding array.*

The following procedure is for processing any one of the arrays in the kit.

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. Blocking & Incubation

2. Add 100 μ l Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.
3. Decant buffer from each well. Add 100 μ l of sample 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 50-500 µg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation, especially if less than 70 µl of sample or reagent is used.

4. 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 shaking. 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 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. 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.

C. Incubation with Biotinylated Antibody Cocktail & Wash

5. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.
6. 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

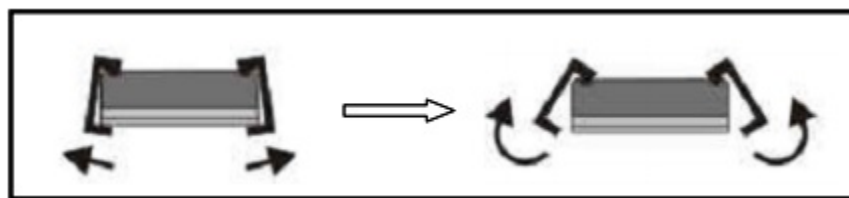
- Decant the samples from each well, and wash 5 times (5 mins each) with 150 μ l of 1X Wash Buffer I and then 2 times with 150 μ l of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

D. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash

- After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
- 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 dark room. Incubate at room temperature for 1 hour.
- Decant the samples from each well, and wash 5 times (5 mins each) with 150 μ l of 1X Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

E. Fluorescence Detection

- 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.

- 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.
- 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.

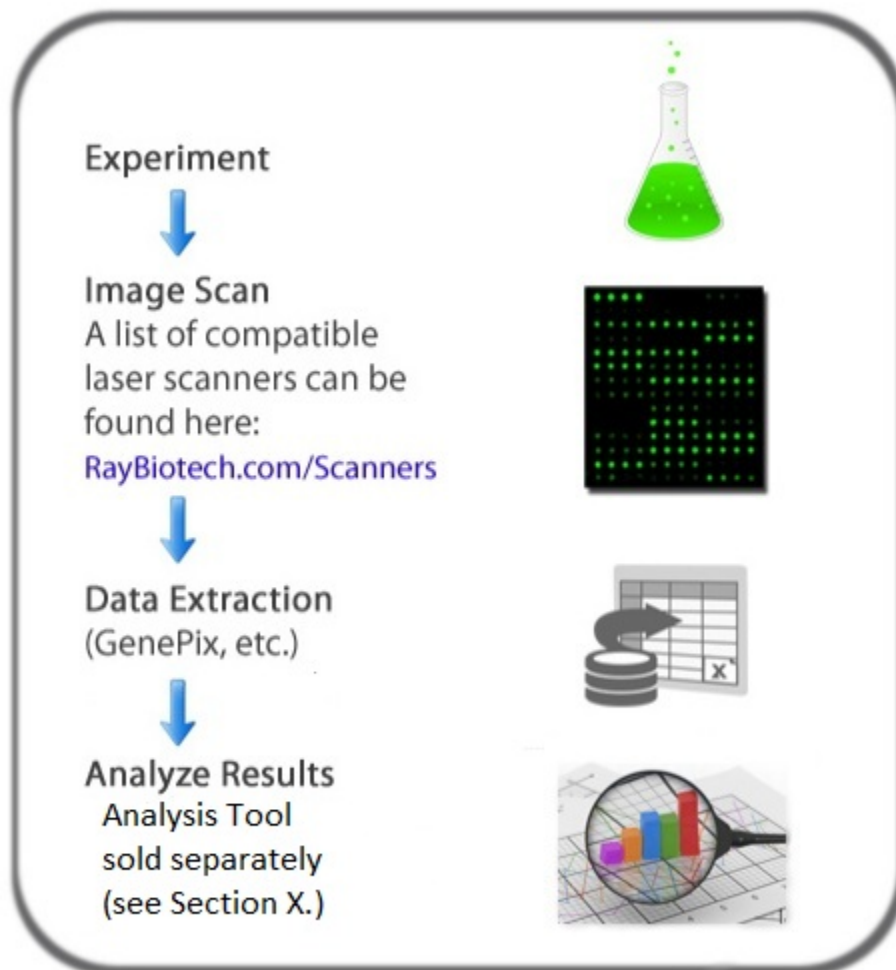
14. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan.

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.

F. Data Analysis

15. >Data extraction can be done using the GAL file that is specific for this array (QAH-CAA-1200) along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). The GAL file can be found on the product web page under the 'Files' tab.

Need help analyzing all that data? All RayBiotech array analysis tools are now free to download! Just like the GAL file, you can find this analysis tool on the product web page under the 'Files' tab. More information can be found in Section X.



IX. Array Map

QAH-INF-3

(hINF-3 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				BLC (CXCL13)			
B	Eotaxin-1 (CCL11)				Eotaxin-2 (MIPF-2)				G-CSF			
C	GM-CSF				I-309				ICAM-1 (CD54)			
D	IFN gamma				IL-1 alpha				IL-1 beta			
E	IL-1ra (IL-1 F3)				IL-2				IL-4			
F	IL-5				IL-6				IL-6sR			
G	IL-7				IL-8				IL-10			
H	IL-11				IL-12p40				IL-12p70			
I	IL-13				IL-15				IL-16			
J	IL-17				MCP-1 (CCL2)				M-CSF			
K	MIG (CXCL9)				MIP-1 alpha (CCL3)				MIP-1 beta (CCL4)			
L	MIP-1 delta (CCL15)				PDGF-BB				RANTES (CCL5)			
M	TIMP-1				TIMP-2				TNF alpha			
N	TNF beta				TNF RI				TNF RII			

QAH-GF-1

(hGF-1 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AR			
B	BDNF				bFGF				BMP-4			
C	BMP-5				BMP-7				beta-NGF			
D	EGF				EGF R				EG-VEGF			
E	FGF-4				FGF-7 (KGF)				GDF-15			
F	GDNF				Growth Hormone (GH)				HB-EGF			
G	HGF				IGFBP-1				IGFBP-2			
H	IGFBP-3				IGFBP-4				IGFBP-6			
I	IGF-1				Insulin				MCF R			
J	NGFR (TNFSR16)				NT-3				NT-4			
K	Osteoprotegerin (OPG)				PDGF-AA				PIGF (PLGF)			
L	SCF				SCF R (CD117)				TGF alpha			
M	TGF beta 1				TGF beta 3				VEGF-A (VEGF)			
N	VEGF R2				VEGF R3				VEGF-D			

QAH-CHE-1

(hCHE-1 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				6Ckine (CCL21)			
B	Axl				Betacellulin (BTC)				CCL28 (MEC)			
C	CTACK (CCL27)				CXCL16				ENA-78 (CXCL5)			
D	Eotaxin-3 (CCL26)				GCP-2 (CXCL6)				GRO			
E	HCC-1 (CCL14)				HCC-4 (CCL16)				IL-9			
F	IL-17F				IL-18 BP alpha				IL-28A			
G	IL-29				IL-31				IP-10 (CXCL10)			
H	I-TAC (CXCL11)				LIF				LIGHT (TNFSF14)			
I	Lymphotactin				MCP-2 (CCL8)				MCP-3 (CCL7)			
J	MCP-4 (CCL13)				MDC (CCL22)				MIF			
K	MIP-3 alpha				MIP-3 beta				MPIF-1 (CCL23)			
L	MSP				NAP-2 (CXCL7)				Osteopontin (OPN)			
M	PARC (CCL18)				Platelet Factor 4 (PF4)				SDF-1 alpha			
N	TARC (CCL17)				TECK (CCL25)				TSLP			

QAH-REC-1

(hREC-1 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				4-1BB (CD137)			
B	ALCAM (CD166)				B7-1 (CD80)				BCMA (TNFRSF17)			
C	CD14				CD30 (TNFRSF8)				CD40 Ligand			
D	CEACAM-1				DR6 (TNFRSF21)				Dkk			
E	Endoglin (CD105)				ErbB3				E-Selectin			
F	Fas				Flt-3 Ligand				GITR (TNFRSF18)			
G	HVEM (TNFRSF14)				ICAM-3 (CD50)				Contactin-2			
H	IL-1 RI				IL-2 R gamma				IL-10 R beta			
I	IL-17R				IL-21 R				LIMPII			
J	Lipocalin-2 (NGAL)				L-Selectin (CD62L)				LYVE-1			
K	MICA				MICB				NRG1-beta 1			
L	PDGF R beta				PECAM-1 (CD31)				RAGE			
M	TIM-1 (KIM-1)				TRAIL R3				Trappin-2			
N	uPAR				VCAM-1				XEDAR			

QAH-CYT-4

(hCYT-4 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin A			
B	AgRP				Angiogenin (ANG)				Angiopoietin-1 (ANG-1)			
C	Angiostatin				Cathepsin S				CD40			
D	Cripto-1				DAN				DKK-1			
E	E-Cadherin				EpcAM (TROP1)				Fas Ligand (TNFSF6)			
F	Fc gamma RIIB/C				Follistatin				Galectin-7			
G	ICAM-2 (CD102)				IL-13 R1				IL-13 R alpha 2			
H	IL-17B				IL-2 R alpha				IL-2 R beta			
I	IL-23				LAP/TGF beta 1				NrCAM			
J	PAI-I				PDGF-AB				Resistin			
K	SDF-1 beta				sgp130				Shh N			
L	Siglec-5 (CD170)				ST2 (IL-1 R4)				TGF-beta 2			
M	Tie-2				Thrombopoietin (TPO)				TRAIL-R4			
N	TREM-1				VEGF-C				VEGF-R1			

QAH-CYT-5

(hCYT-5 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Adiponectin (ACRP30)			
B	Adipsin				Alpha-fetoprotein (AFP)				ANGPTL4			
C	Beta-2 Microglobulin (B2M)				BCAM				CA125			
D	CA15-3				CEA				CRP			
E	ErbB2				Ferritin				FSH			
F	GRO alpha (CXCL1)				HCG beta (HCGb)				IGF-I R			
G	IL-1 RII				IL-3				IL-18 R beta			
H	IL-21				Leptin				MMP-1			
I	MMP-2				MMP-3				MMP-8			
J	MMP-9				MMP-10				MMP-13			
K	NCAM-1 (CD56)				Nidogen-1				NSE			
L	Oncostatin M (OSM)				Procalcitonin (PCT)				Prolactin			
M	PSA-free				Siglec-9				TACE			
N	Thyroglobulin				TIMP-4				TSH			

QAH-CYT-6

(hCYT-6 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				2B4 (CD244)			
B	ADAM-9				Angiopoietin-2 (ANG-2)				APRIL			
C	BMP-2				BMP-9				C5a			
D	Cathepsin L				CD200				CD97			
E	Chemerin				Dcr3				FABP2			
F	FAP				FGF-19				Galectin-3			
G	HGF R				IFN alpha/beta R2				IGF-II			
H	IGF-II R				IL-1 R6 (IL-1 Rrp2)				IL-24			
I	IL-33 (IL-1 F11)				Kallikrein 14				Legumain			
J	LOX-1				MBL				Nepilysin			
K	Notch-1				NOV (CCN3)				Osteoactivin			
L	PD-1				PGRPs				Serpins A4			
M	sFRP-3				Thrombomodulin				TLR2			
N	TRAIL R1				Transferrin				WIF-1			

QAH-CYT-7

(hCYT-7 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ACE-2			
B	Albumin				AMICA				Angiopoietin-4 (ANG-4)			
C	BAFF				CA19-9				CD163			
D	Clusterin				CRTAM				CXCL14 (BRAK)			
E	Cystatin C				Decorin				Dkk-3			
F	DLL1				Fetuin A				aFGF (FGF-1)			
G	FOLR1				Furin				GASP-1			
H	GASP-2				G-CSF R (CD114)				HAI-2			
I	IL-17B R (IL-17 RB)				IL-27				LAG-3			
J	LDL R				Pepsinogen I (PG1)				RANK			
K	RBP4				SOST				Syndecan-1			
L	TACI				TFPI				Thrombospondin 1			
M	TRAIL R2				TRANCE				Troponin I			
N	uPA				VE-Cadherin (CDH5)				WISP-1 (CCN4)			

QAH-CYT-8

(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-9

(hCYT-9 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAMTS13			
B	Aggrecan				Angiotensinogen (AGT)				B7-H1 (CD274)			
C	BMPR-IA (ALK-3)				BMPR-II				Cadherin-11			
D	CD27 (TNFRSF7)				CD6				Ck beta 8-1 (CCL23)			
E	CNTF				DNAM-1 (CD226)				EMMPRIN (CD147)			
F	FLRG				Follistatin-like 1 (FSL1)				Fractalkine (CX3CL1)			
G	Galectin-1				GITR Ligand				Granulysin (LAG-2)			
H	IL-1 R3 (IL-1 R Acp)				IL-15 R alpha				IL-17E (IL-25)			
I	IL-32 alpha				L1CAM-2 (CHL-1)				LRIG3			
J	LRP-6				MEPE (OF45)				Nectin-4			
K	Periostin				Persephin				Renin			
L	RGM-B				ROBO3				S100A8			
M	Siglec-7 (CD328)				Syndecan-3				Thrombospondin 2			
N	Thrombospondin 5				Tie-1				ULBP-2			

QAH-CYT-10

(hCYT-10 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAM8			
B	ADAM12				B7-H3 (CD276)				BMPR-IB			
C	Cadherin-4				Cadherin-13				CD48 (SLAMF2)			
D	CD58 (LFA-3)				CD84 (SLAMF5)				CD99			
E	CD155 (PVR)				CD229 (SLAM3)				CEACAM-5			
F	CF XIV				Cystatin A				Cystatin B			
G	Cystatin E/M				Desmoglein 2				DR3 (TNFRSF25)			
H	ErbB4 (HER4)				ESAM				FGF-21			
I	Galectin-2				Galectin-9				ICOS			
J	JAM-A (CD321)				JAM-B (CD322)				Kallikrein 5			
K	Midkine				Pentraxin 3				Pref-1 (DLK-1)			
L	Siglec-10				SLAM (CD150)				SP-D			
M	Syndecan-4				Testican 2 (SPOCK2)				TIM-3 (KIM-3)			
N	TLR4				TRAIL (TNFSF10)				ULBP-1			

QAH-CYT-11

(hCYT-11 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ALK-1			
B	B7-H2				BLAME				BMP-8			
C	CD28				Common beta Chain				Contactin-1			
D	Desmoglein-1				Desmoglein-3				EDAR			
E	EphA1				EphB6				Ephrin-B3			
F	Epiregulin				FGF-12				FGF-17			
G	FOLR2				Galectin-8				GHR			
H	Glypican 1				Glypican 5				IFN-gamma R1			
I	IL-22 R alpha 1				IL-22BP				IL-23 R			
J	IL-31 RA				IL-7 R alpha				Integrin alpha 5			
K	MDM2				Nectin-1				NKp30			
L	Nogo Receptor				Notch-3				OSM R beta			
M	Prolactin R				RELTL				Ryk			
N	Semaphorin 6D				Semaphorin 7A				Siglec-11			

QAH-CYT-12

(hCYT-12 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				B7-2			
B	BAFF R				Calcitonin				Calsyntenin-1			
C	Cathepsin E				cIAP-2				Coagulation Factor VII			
D	Complement MASP3				Endocan				EphA2			
E	EphB4				Ephrin-A4				FGF-23			
F	FGF-5				Flt-3				GLP-1			
G	Glypican 2				GM-CSF R alpha				GP73			
H	HTRA2				IL-20 R alpha				IL-4 R alpha			
I	JAM-C				Luteinizing hormone (LH)				Matrilin-3			
J	Meprin alpha				MSP R				N-Cadherin			
K	Nephrilysin-2				NKp44				PAPP-A			
L	Pepsinogen II				Presenilin 1				PTH			
M	PYY				SOX2				TFF3			
N	TFPI-2				TRACP				Ubiquitin+1			

QAH-CYT-13

(hCYT-13 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ACE			
B	Activin RIB				ADAM23				Artemin			
C	Cardiotrophin-1				Cathepsin V				FABP1			
D	FGF-20				GDF-8				HAI-1			
E	IL-27 R alpha				Insulin R				Kallikrein 7			
F	LIF R alpha				Lipocalin-1				LTbR			
G	Mesothelin				MFRP				Neuropilin-2			
H	Neurturin				Nidogen-2				Olfactomedin-2			
I	p53				PD-ECGF				PDGF-CC			
J	Progranulin				Ret				ROBO4			
K	Semaphorin 6B				Serpins F1				SREC-I			
L	SREC-II				TLR1				TLR3			
M	TPP1				TREM-2				TrkC			
N	TROY				Uromodulin				XIAP			

QAH-CYT-14

(hCYT-14 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				4-1BB Ligand			
B	Activin RIB				Aminopeptidase P2				BAMBI			
C	BOC				Brevican				Carbonic Anhydrase XII			
D	Carboxypeptidase A2				CD300c				CD320			
E	CDNF				CDO				CHST1			
F	CHST4				CILP-1				CNTF R alpha			
G	CRIM1				CRTAC1				CXADR			
H	Dopa Decarboxylase				DPPII				DSPG3			
I	EMR2				FCAR				FCRL1			
J	FCRL2				Gas6				GPR56			
K	GPVI				Hepsin				ILT2			
L	Jagged 2				Kirrel3				KLF4			
M	LAIR1				LAMP				LAMP1			
N	MDGA1				MIS RII				Neurexin 3 beta			

QAH-CYT-15

(hCYT-15 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AMIGO			
B	Aminopeptidase LRAP				Amnionless				Arylsulfatase A			
C	Bcl-w				CD109				CD157			
D	CD34				CD83				CLEC-1			
E	CLEC10A				CMG-2				CREG			
F	Cystatin SN				Cytokeratin-8				Dectin-1			
G	Desmocollin-3				Endoglycan				Galectin-4			
H	HAPLN1				Jagged 1				Langerin			
I	Lumican				Matriptase				MEP1B			
J	Nectin-3				OX40				OX40 Ligand			
K	p27				Pappalysin-2				Plexin B3			
L	Plexin D1				proGRP				PSA-total			
M	Reg1B				RGM-A				ROBO2			
N	Spinesin				TWEAK R				ULBP-3			

QAH-CYT-16

(hCYT-16 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin RIIA			
B	Biglycan				CA13				CA2			
C	CA72-4				CLEC-2				C-myc			
D	Cystatin D				Erythropoietin				FCRL5			
E	FGF-16				GATA-4				GFR alpha-1			
F	GFR alpha-2				Granzyme B				Granzyme H			
G	HIF-1a				hPAPP-A				IFNb			
H	IL-17 RC				IL-19				IL-20 R beta			
I	IL-22				ILT4				LAIR2			
J	LSECTin				Netrin-4				Norrin			
K	NRG1a				PD-L2				PDX-1			
L	Podocalyxin				RGM-C				S100A1			
M	Semaphorin 6A				SLITRK5				SR-AI			
N	ST6GAL1				Thyroid Peroxidase				Troponin C			

QAH-CYT-17

(hCYT-17 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin RIA			
B	ASAH1				B4GalT1				BAI1			
C	Brorin				C1qTNF4				CA14			
D	CA4				CA6				CA8			
E	Cadherin-6				Casp2				CD27 Ligand			
F	CD300a				CD300e				CD300f			
G	CD4				CD5				CD69			
H	CK18				CK19				CPB1			
I	CRISP-2				DDR1				FUT8			
J	MIA				NTAL				NTB-A			
K	OMgp				PEAR1				Podoplanin			
L	PTH1R				Reg4				ROR1			
M	Semaphorin 4G				Serpins A5				Serpins B6			
N	Siglec-1				Sirtuin 2				Sirtuin 5			

QAH-CYT-18

(hCYT-18 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ANGPTL7			
B	CD36				CLEC9a				CL-P1			
C	Dectin-2				DLL4				DSCAM			
D	EDIL3				ENPP-7				Enteropeptidase			
E	FCRL3				FCRLB				FGF-3			
F	FLRT1				FLRT2				GBA3			
G	GDF-11				Glycoprotein V				Granzyme A			
H	IGSF4B				IL-28 R alpha				Kynureninase			
I	LAMA4				LRRC4				LRRTM3			
J	NG2				NQO-1				PCSK2			
K	PILR-alpha				Plexin A4				POGLUT1			
L	PRELP				Smad4				SOX15			
M	SOX7				SOX9				Syntaxin 6			
N	TROP-2				TSLP R				UNC5H4			

QAH-CYT-19

(hCYT-19 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAM22			
B	ARSB				B3GNT2				CA5B			
C	Caspase 7				Caspase 8				CD11b			
D	CD172g				CD39L2				CD39L4			
E	CD49b				CD7				CEACAM-3			
F	CPE				FABP6				FAM3C			
G	GDF-3				GSTM1				Kallikrein 11			
H	Kallikrein 12				Kremen-2				OSCAR			
I	PTP1B				Reg3A				R-Spondin 2			
J	S100A13				Semaphorin 4C				Sirtuin 1			
K	SMPD1				Sortilin				SPINK1			
L	Stabilin-2				SULT2A1				TCN2			
M	THSD1				TrkA				UCH-L3			
N	VAP-A				vWF-A2				Wnt-4			

QAH-CYT-20

(hCYT-20 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAMTSL-1			
B	AMSH				Annexin V				BATF3			
C	Bora				Cadherin-17				Caveolin-2			
D	CD2				CD200 R1				CHST3			
E	COMT				Cystatin SA				DBH			
F	Desmin				EXTL3				Ficolin-1			
G	FosB				FRS2				GATA-5			
H	GFAP				GLI-3				HepaCAM			
I	HIF-1 beta				HSD17B1				IDO			
J	Kallikrein 1				Kell				MDL-1			
K	NPDC-1				Numb				Olig2			
L	p63				Pax3				Semaphorin 4D			
M	SPHK1				TAZ				TC-PTP			
N	TGM3				TPST2				TREML1			

QAH-CYT-21

(hCYT-21 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Cf10			
B	CHMP2B				Contactin-3				Cortactin			
C	Crkl				Cyr61				DAPP1			
D	DCTN1				DFF45				DRAK1			
E	GRAP2				GRK5				HAO-1			
F	LRIG1				MMP-12				NCK1			
G	Nectin-2				Nesfatin-1				Neurogranin			
H	Nfr2				NUDT5				NUP85			
I	PAR1				PP				PRX2			
J	PSMA1				PU.1				RalA			
K	RCOR1				Serpine B8				SH2D1A			
L	SHP-1				Siglec-6				SorCS3			
M	THAP11				ULBP-4				UNC5H3			
N	VAMP-1				VAMP-2				Visfatin			

QAH-CYT-22

(hCYT-22 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				C1qTNF9			
B	CA5A				CANT1				Cathepsin H			
C	Contactin-5				CTRC				Draxin			
D	EphB2				EphB3				FABP8			
E	Fgr				FKBP51				FUCA1			
F	Galanin				GALNT10				GKN1			
G	Glyoxalase II				HS3ST1				HS3ST3B1			
H	Lin28				LOXL2				LRRTM4			
I	MAP1D				Matrilin-2				MCEMP1			
J	Mcl-1				MDGA2				MEF2C			
K	METAP2				Neurocan				Nogo-A			
L	PCK1				PIGF-2				PON1			
M	SALM4				Semaphorin 6C				SorCS2			
N	ST3GAL1				ST8SIA1				TSK			

QAH-CYT-23

(hCYT-23 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADA			
B	AIF				AKR1C4				ASAH2			
C	BCL-2				BID				Calreticulin			
D	Calreticulin-2				CD314				CD39L3			
E	CD51				CD99-L2				CDC25B			
F	Cerberus 1				CHST2				Cochlin			
G	CRELD2				DC-SIGNR				eNOS			
H	ENPP-2				FABP4				FcERI			
I	FGF R5				GALNT2				GALNT3			
J	GIF				GPR111				GUSB			
K	Inhibin A				LILRB4				Neuroglycan C			
L	NKp46				NPTXR				ROR2			
M	SCCA2				Siglec-2				SIRP alpha			
N	SorCS1				Trypsin 1				Trypsin 3			

QAH-CYT-24

(hCYT-24 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AMIGO2			
B	Arginase 1				B7-H4				Bcl-10			
C	CD42b				CD73				CES1			
D	CES2				cAP-1				Cyclophilin A			
E	Cystatin S				DNMT3A				Epimorphin			
F	GDF-9				Glypican 3				GPR115			
G	HE4				HO-1				HS3ST4			
H	IGSF3				IL-17 RD				Integrin alpha 1			
I	KIR2DL3				LAMP2				LEDGF			
J	MOG				Nestin				Neudesin			
K	Neuroigin 2				NKp80				Osteoadherin			
L	PDGF R alpha				PRDX4				Syntaxin 4			
M	TFAFA1				TFAFA2				TFAFA5			
N	Tenascin R				TGM4				TMEFF1			

QAH-CYT-25

(hCYT-25 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ACP6			
B	Annexin A4				ASAM				BAI3			
C	BAK				Brachyury				BTLA			
D	Caspase-1				CD160				CDK5 Activator			
E	Complexin-1				Cyclin E1				DC-SIGN			
F	DLEC				Doc2 alpha				ER alpha			
G	FGF R3				FKBP38				FUT11			
H	FXR				Galectin-10				GLB1			
I	Glucokinase				GMF-beta				GSK-3 beta			
J	HNMT				IKK gamma				IRF2			
K	ISLR-2				JunB				Latexin			
L	LMW-PTP				MPF				NFKB1			
M	Olig3				p130Cas				PAG1			
N	PDCD6				Pro-EGF				PTP-MEG2			

QAH-CYT-26

(hCYT26 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin AB			
B	APLP-1				Caspase-9				CD286			
C	CD44				CDX4				CHRD2			
D	CIB1				Collagen XIII				DAB2			
E	DC-LAMP				DEFB-3				DSCAM-L1			
F	FoxM1				FoxO3				FoxP3			
G	F-Spondin				ILKAP				Integrin alpha 4			
H	LRP-1 Cluster III				LRPAP				LRRTM1			
I	Melan-A				mGluR3				MKK6			
J	NgR3				Nicastrin				Olig1			
K	Pax4				Paxillin				Relaxin-1			
L	R-Spondin 3				Semaphorin 3E				Serp in B2			
M	SLITRK4				SNCG				TBX2			
N	TRAF-3				TSC1				USAG1			

QAH-CYT-27

(hCYT27 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				15-PGDH			
B	ALPL				ANGPTL2				Annexin A1			
C	Attractin				Catalase				Cathepsin C			
D	CD47				CD79A				CHIT1			
E	CLUL1				CNDP1				CNP			
F	COCO				ELF3				FABP3			
G	FABP5				Fibromodulin				FLRT3			
H	FOLR3				GFR alpha-3				GSTP1			
I	IDS				Ikb- alpha				JNK2			
J	KLK6				KLK8				NEL1			
K	NTH1				OBCAM				PRX1			
L	SCCA1				Serp in A12				Sirtuin 3			
M	SOD1				SOX10				SULT1B1			
N	SULT1C2				SULT2B1				TIGIT			

QAH-CYT-28

(hCYT28 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin C			
B	AHCY				APE				Bax			
C	BNIP3				Caspase-3				Cathepsin F			
D	CD177				CD96				CLEC4D			
E	C-Peptide				DCBLD2				Dystroglycan			
F	FAM3A				GCNT1				GLA			
G	HAPLN4				hCG alpha				HMGB3			
H	Ikb-E				IRF4				KIR3DL1			
I	Klotho beta				Lck				LILRA5			
J	METTL11A				p55PIK				PCDH17			
K	PLA2G4A				RalB				S100A2			
L	S100B				SH2B1				Siglec-3			
M	SOD3				ST8SIA2				Testican 1			
N	TRP14				VAP-B				ZAP70			

QAH-SAP-4

(hSAP-4 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Azurocidin			
B	BLMH				C1q R1				C1qTNF1			
C	C2				Cathepsin A				Cathepsin D			
D	Cathepsin X				CD13				CD35			
E	Contactin-4				CPA1				CPM			
F	DNER				Endorepellin				Fetuin B			
G	Hepassocin				LEKT1				MAGP-2			
H	MCAM				MMR				Neuropilin-1			
I	PAM				Park7				PCSK9			
J	PLUNC				PRCP				PRTN3			
K	P-Selectin				PSP94				S100A6			
L	SAA				SLPI				SPARCL1			
M	Tenascin C				Thioredoxin-1				TIM-4			
N	Trypsin Pan				TSP-4				VSIg4			

X. Array Data Analysis Tool

The RayBio Analysis Tools are array specific, Excel-based program that perform sophisticated data analysis on the raw numerical data extracted from the array scan. All RayBiotech array analysis tools are now free to download! Just like the GAL file, you can find this analysis tool on the product web page under the 'Files' tab.

Key features:

- Simplicity: Easy to operate and requires no professional training. With a simple copy and paste process, the cytokine expression levels are determined per sample.
- 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.
- User Intervention: The program allows for user manual handling of outliers and other analytical data.
- Analyze Multiple Slide: The data for multiple slides can be inputted for easy slide-to-slide comparison.

XI. 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/shaking during incubations. check for bubble formation and remove bubbles.
	Arrays are not completely covered by reagent	Completely cover arrays with solution for all required steps.
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
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.

XII. Select 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. *Pharmaceutical 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 Ethnopharmacology*. 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. *Alzheimers 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

Note: The citations listed above are for the Quantibody® product line, which is the same as the GS-Series, but include protein standards for quantitation.

XIII. Experiment Record Form

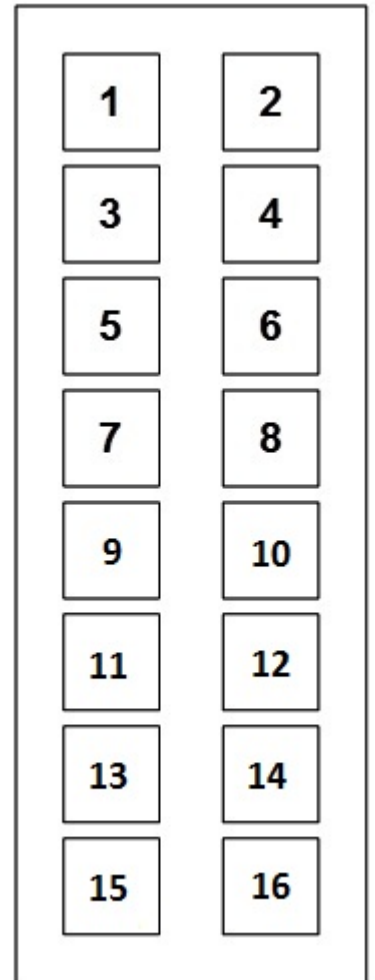
Date: _____

File Name: _____

Laser Power: _____

PMT: _____

Well No.	Sample Name	Dilution factor
1		
2		
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16		



XIV. How to Choose a GS-Series Array?

Species-based selection:

Human (GSH-)	Mouse (GSM-)	Rat (GSR-)	Bovine (GSB-)	Canine (GSC-)
Equine (GSE-)	Feline (GSF-)	Ovine (GSO-)	Primates (GSN-)	Porcine (GSP-)
Rabbit (GSL-)				

Function-based selection:

Adhesion Molecule Arrays	Angiogenesis Arrays	Bone Metabolism Arrays	Chemokine Arrays
Cancer Biomarker 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 GS-Series & Quantibody[®] platform to detect 1,200 human, 640 mouse, 282 rat, 50 porcine, or 30 bovine proteins. GLP-Compliant testing services are also available.

This product is for research use only.



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