G-Series Mouse Cytokine Antibody Array 640

A combination of 16 non-overlapping arrays to measure the relative expression levels of 640 Mouse cytokines

Catalog #: GSM-CAA-640

User Manual
Last revised October 1, 2021

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (640)	Arrays Included: GSM-CYT-4 (40); GSM-CYT-5 (40); GSM-CYT-6 (40); GSM-CYT-7 (40); GSM-CYT-8 (40); GSM-CYT-9 (40); GSM-CYT-10 (40); GSM-CYT-11 (40); GSM-CYT-12 (40); GSM-CYT-13 (40); GSM-CYT-14 (40); GSM-CYT-15 (40); GSM-CYT-16 (40); GSM-CYT-17 (40); GSM-CYT-18 (40); GSM-CYT-19 (40) *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

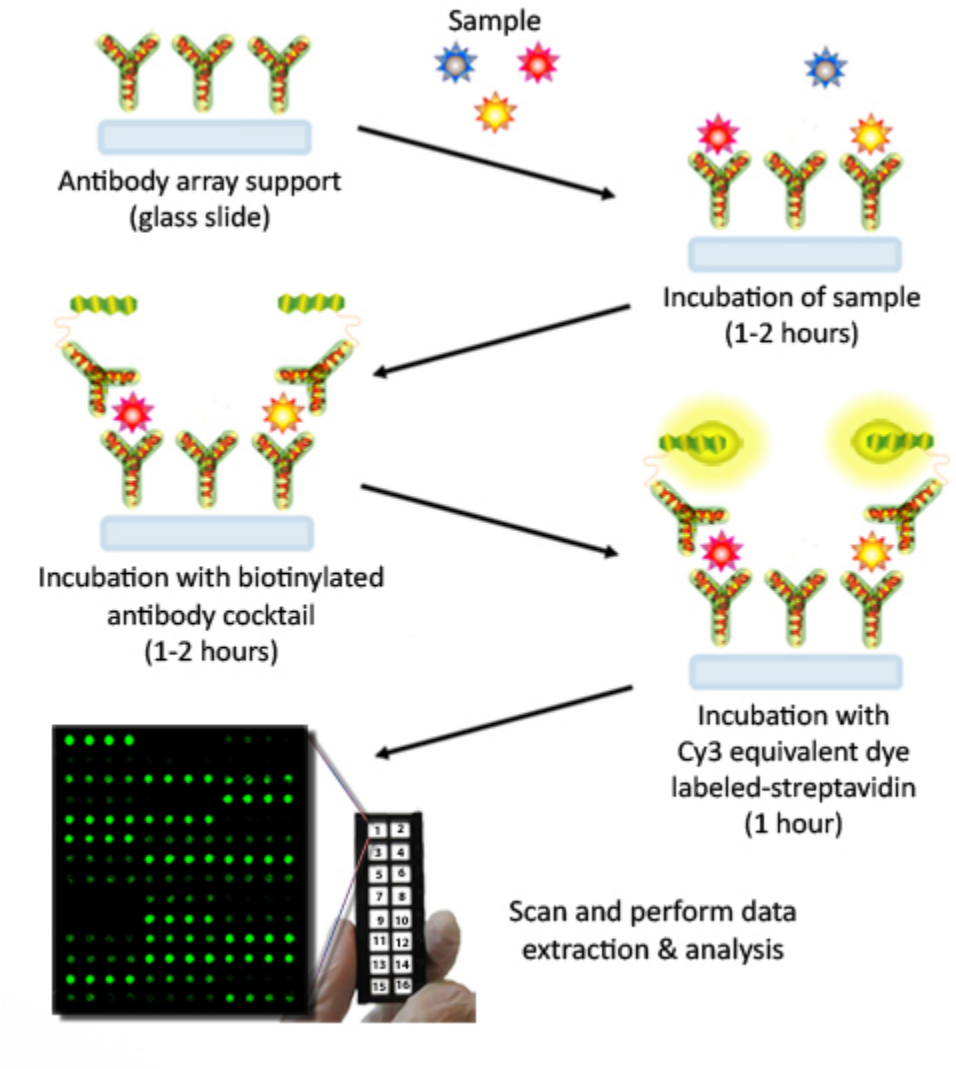
II. Introduction

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. 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 µl	2 x 1-25 µl
6	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 µl	2 x 5 µl
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 µl 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 µg/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 µl, or 500 µg 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 µl of sample or reagent is used.
- Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

VIII. Protocol

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

3. Decant buffer from each well. Add 100 µl 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 ul 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

7. 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 shaking. Completely remove wash buffer in each wash step.

D. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash

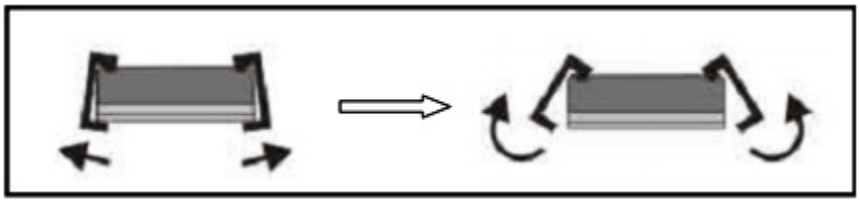
8. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

9. 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.

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

E. Fluorescence Detection

11. 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.

12. 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.

13. 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 N_2 stream.

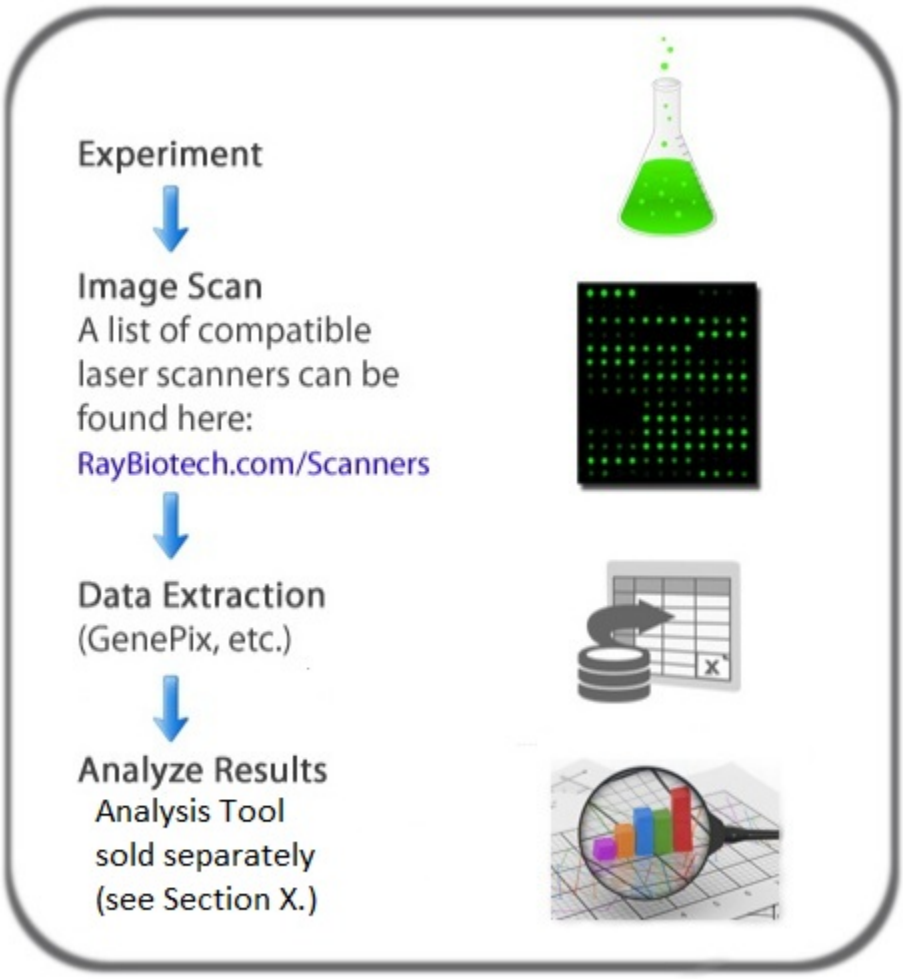
14. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan.

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 (QAM-CAA-640) 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

QAM-CYT-4

Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Amphiregulin			
B	Axl				CD27 Ligand				CD30 (TNFRSF8)			
C	CD40 (TNFRSF5)				CXCL16				EGF			
D	E-Selectin				Fractalkine				GITR (TNFRSF18)			
E	HGF				IGFBP-2				IGFBP-3			
F	IGFBP-5				IGFBP-6				IGF-1			
G	IL-12 p70				IL-17E (IL-25)				IL-17F			
H	IL-1 ra (IL-1 F3)				IL-2 R alpha				IL-20			
I	IL-23 p19				IL-28A				I-TAC (CXCL11)			
J	MDC (CCL22)				MIP-2				MIP-3 alpha (CCL20)			
K	Osteopontin (SPP1)				Osteoprotegerin				Prolactin			
L	Pro-MMP-9				P-Selectin				Resistin			
M	SCF				SDF-1 alpha				Thrombopoietin (TPO)			
N	VCAM-1 (CD106)				VEGF-A				VEGF-D			

QAM-CYT-5

Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				bFGF			
B	BLC (CXCL13)				CD30 Ligand				Eotaxin-1 (CCL11)			
C	Eotaxin-2 (MPIF-2)				Fas Ligand (TNFSF6)				GCSF			
D	GM-CSF				ICAM-1 (CD54)				IFN-gamma			
E	IL-1 alpha				IL-1 beta				IL-2			
F	IL-3				IL-4				IL-5			
G	IL-6				IL-7				IL-10			
H	IL-12 p40				IL-13				IL-15			
I	IL-17A				IL-21				KC (CXCL1)			
J	Leptin				LIX				MCP-1 (CCL2)			
K	MCP-5				M-CSF				MIG (CXCL9)			
L	MIP-1 alpha (CCL3)				MIP-1 gamma				Platelet Factor 4			
M	RANTES (CCL5)				TARC (CCL17)				I-309 (CCL1)			
N	TNF RI (TNFRSF1A)				TNF RII (TNFRSF1B)				TNF-alpha			

QAM-CYT-6

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	**Ace**				**ALK-1**				**Cardiotriohin-1 (CT-1)**			
C	**CD27 (TNFRSF7)**				**CD40 Ligand**				**CTLA-4 (CD152)**			
D	**Decorin**				**DKK-1**				**Dtk**			
E	**Endoglin (CD105)**				**Fc-gamma-RIIB**				**Flt-3 Ligand**			
F	**Galectin-1**				**Galectin-3**				**Gas 1**			
G	**Gas 6**				**GITR Ligand**				**HAI-1**			
H	**HGFR**				**IL-1 R4 (ST2)**				**IL-3 R beta**			
I	**IL-9**				**JAM-A (CD321)**				**Leptin R**			
J	**L-selectin (CD62L)**				**Lymphotactin**				**MadCAM-1**			
K	**MFG-E8**				**MIP-3 beta (CCL19)**				**Neprilysin**			
L	**Pentraxin-3 (TSG-14)**				**RAGE**				**TACI**			
M	**TREM-1**				**TROY**				**TSLP**			
N	**TWEAK R**				**VEGF R1**				**VEGF R3**			

QAM-CYT-7

Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				CD80 (B7-1)			
B	BAFF R				Betacellulin (BTC)				C5a			
C	CCL6				CD48 (SLAMF2)				CD6			
D	Chemerin				Clusterin				CXCL15			
E	Cystatin C				DAN				DLL4			
F	EDAR				Endocan				Fetuin A			
G	H60				IL-33				IL-7 R alpha			
H	Kremen-1				Limitin				Lipocalin-2 (NGAL)			
I	LOX-1				Marapsin				MBL-2			
J	Meteorin				Nope				NOV (CCN3)			
K	Osteoactivin				OX40 Ligand				P-Cadherin			
L	Periostin				PIGF-2				Progranulin			
M	Prostasin				Renin 1				Testican 3			
N	TIM-1 (KIM-1)				TRAIL (TNFSF10)				Tryptase epsilon			

QAM-CYT-8

Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				6Ckine			
B	Activin A				ADAMTS1				Adiponectin			
C	ANG-3				ANGPTL3				Artemin			
D	CCL28				CD36				Chordin			
E	CRP				E-Cadherin				Epigen			
F	Epiregulin				Fas				Galectin-7			
G	gp130				Granzyme B				Gremlin			
H	IFN-γ R1				IL-17B				IL-17B R			
I	IL-22				MIP-1β				MMP-2			
J	MMP-3				MMP-10				PDGF-AA			
K	Persephin				sFRP-3				Shh-N			
L	SLAM				TCK-1				TECK			
M	TGFβ1				TRANCE				TremL1			
N	TWEAK				VEGF-B				VEGF-R2			

QAM-CYT-9

(mCYT9 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				2B4			
B	4-1BB Ligand				Activin RIB				Ameloblastin			
C	ANGPTL7				B7-2				B7-H2			
D	B7-H3				B7-H4				BAFF			
E	C1q R1				Cathepsin H				CD117			
F	CD157				CD200				CD229			
G	CD28				CD300b				CD39			
H	CD44				CD45				CD69			
I	CD99-L2				CHL-1				CHRDL2			
J	COCO				CRACC				CXADR			
K	DcTRAIL R1				Dectin-2				DNAM-1			
L	DNER				Endoglycan				EphA8			
M	EphB2				EphB4				EphB6			
N	Ephrin-A2				Ephrin-A4				FGF-21			

QAM-CYT-10

(mCYT10 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAM9			
B	CD14				CD39L3				CDNF			
C	Cripto				CXCL14				Epimorphin			
D	Erythropoietin R				Flt-3				Follistatin			
E	Frizzled-1				Frizzled-4				FSTL1			
F	GDF-3				GDF-7				GFR alpha-3			
G	IFN-gamma R2				IL-1 RI				IL-1 RII			
H	IL-10 R beta				IL-11 R alpha				IL-15 R alpha			
I	IL-17 RA				IL-17 RC				IL-20 R beta			
J	IL-1F8				IL-21 R				IL-23 R			
K	IL-28A				JAM-C				Klotho beta			
L	Layilin				LDL R				LIF			
M	Matrilin-3				Lymphotoxin beta R				Nephrin			
N	Neurocan				NKp46				Laminin alpha 4			

QAM-CYT-11

(mCYT11 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	ADAM15				AFP				ASAHL			
C	C4.4A				CA12				CA14			
D	CA4				CA9				Cadherin-4			
E	CD2				CD4				CD90			
F	CDCP1				CEACAM-1				CLEC9a			
G	CFVII				Contactin-4				Contactin-6			
H	EpCAM				EphA5				FCRL1			
I	FGF-10				FGF-4				FGF-6			
J	FLRG				G-CSF R				GPV			
K	GPVI				HDAC8				HS6ST3			
L	IGF-II				IGSF8				IL-1 R6			
M	IL-1 R7				IL-16				IL-17C			
N	IL-18 BPc				IL-31				IL-34			

QAM-CYT-12

(mCYT12 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ASAM			
B	Cystatin B				DLL1				Kallikrein 7			
C	Kremen-2				LAMP1				LIGHT			
D	LIMPII				LRPAP				LRRC32			
E	Matrilin-2				Mcpt6				MEP1A			
F	MEPE				MESDC2				METRNL			
G	Mimecan				Nectin-2				Neurturin			
H	NGF R				NgR				Olfactomedin-1			
I	Oncostatin M				OSM R beta				Osteoadherin			
J	OX40				PD-1				PDGF R beta			
K	PD-L2				PILR-beta				PLA2G2A			
L	Plexin A1				Plexin C1				Podocalyxin			
M	Podoplanin				Protocadherin-12				Prss34			
N	RANK				Reg2				Relaxin-1			

QAM-CYT-13

(mCYT13 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Ret			
B	RGM-B				RGM-C				ROBO3			
C	SEMA3C				SEMA3F				SEMA4C			
D	SEMA4F				SEMA4G				SEMA6C			
E	Siglec-3				Siglec-E				SIGNR1			
F	Slit2				SMOC-1				SorCS2			
G	SP-D				SR-AI				STC-2			
H	Syndecan-1				Syndecan-3				TFPI-2			
I	TGF-beta RI				TGF-beta RII				TGM2			
J	TIGIT				TIM-3				TLR2			
K	TNFRH3				Tpo R				TRAIL R2			
L	TREM-2				TrkC				TROP-2			
M	Trypsin 3				Tryptase-5				TSLP R			
N	uPAR				VE-Cadherin				Wnt-2b			

QAM-CYT-14

(mCYT14 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				aFGF			
B	ANG-2				APCS				beta-NGF			
C	BMP-5				BMPR-II				CD164			
D	CHST4				CHST7				CRELD1			
E	FCRN				FGF R3				GAPDH			
F	GDF-11				IFNAR1				IFN-beta			
G	IL-2 Rg				LAG-3				Mer			
H	MMP-8				MOG				Neuroplastin			
I	PAI-1				RBP4				Ryk			
J	S100A1				S100A6				S100A9			
K	SEMA6A				Serpin F1				Siglec-1			
L	TCAM-1				Thrombomodulin				Tie-2			
M	TIM-4				TIMP-4				TLR1			
N	TSP-4				VLDL R				VSIG4			

QAM-CYT-15

(mCYT15 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AGT			
B	BCHE				C1QBP				CADM3			
C	CAMK4				CD200R4				CD53			
D	CD59a				CD7				CHST3			
E	CNDP1				CNTN1				CNTN2			
F	CPM				CST7				ENPP2			
G	ERBB2				FCER2				HN1			
H	HPGD				IFNA2				IL36G			
I	Klrb1a				KYNU				LAIR1			
J	MFI2				NAALADL1				NCSTN			
K	PREP				PSMB6				PTK6			
L	PVR				S100A3				SDC4			
M	SerpinA10				Serpina3c				SIRPB1			
N	THOP1				TSC22D1				UCHL1			

QAM-CYT-16

(mCYT16 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ARSA			
B	BDNF				BMP-6				BMP-9			
C	CA2				CD180				CNTF			
D	DR3				EphA6				FGF-23			
E	FGF-8				HAI-2				IL12RB2			
F	IL-27 Ra				Kirrel2				KLRC1			
G	MANF				MCAM				MD-1			
H	MEP1B				Netrin-G1a				NTB-A			
I	PCSK9				PPM1A				RGM-A			
J	R-Spondin 4				SELPLG				SerpinB10			
K	SerpinB8				Siglec-2				Siglec-F			
L	SIRPA				ST6GALNAC2				Tissue Factor			
M	TLR6				Transferrin				TSP-2			
N	VNN1				WISP-1				Wnt-8a			

QAM-CYT-17

(mCYT17 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				BTLA			
B	BTN1A1				C1ra				Cathepsin L			
C	CD200 R1				CD2F-10				CD38			
D	Cerberus 1				CRISP-4				CXCL17			
E	DCC				DcTRAIL R2				Dkk-2			
F	EG-VEGF				EPCR				EphA3			
G	EphA4				Ephrin-B1				Ephrin-B2			
H	Frizzled-2				Galectin-4				Galectin-9			
I	GDF-5				GDF-9				GFR alpha -2			
J	GHR				HIN-1				ICAM-5			
K	IGFBP-L1				IL-10 R alpha				IL-17 RD			
L	IL-22 R alpha 1				IL-3 R alpha				JAM-B			
M	Latexin				LILRC1				Matrilin-4			
N	Netrin-1				Netrin-4				Noggin			

QAM-CYT-18

(mCYT18 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AMICA			
B	APLP-1				Arylsulfatase G				B7-H5			
C	CD47				CES2				CLEC4F			
D	CPA1				CPB1				CREG			
E	CRELD2				Dectin-1				EMMPRIN			
F	FABP4				FCRL5				FGF R5			
G	FOLR1				Galectin-2				Glypican 5			
H	ICOS				IDS				IGFBP-1			
I	IL-12 R beta 1				IL-30				IL-4 R alpha			
J	Lefty-1				Legumain				LRIG1			
K	Mcpt7				MDL-1				MGL2			
L	MSP R				NCAM-1				Netrin-G2a			
M	Neuroglycan C				Nidogen-2				PDGF R alpha			
N	PILR-alpha				PIR-B				Plexin B2			

QAM-CYT-19

(mCYT19 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				A33			
B	Angiogenin				ART4				ASGR1			
C	BAMBI				Bcl-xL				BID			
D	BMP-7				CD160				CD34			
E	CD5				CD74				CD99			
F	CES1				CL-P1				CMG-2			
G	DSPG3				EphA1				EphA7			
H	EphB3				Erythropoietin				FAM3C			
I	FDPS				FGF R4				Frizzled-9			
J	GDF-8 Propeptide				IGSF4A				IL-1F6			
K	IL-5 R alpha				IP-10				JNK1			
L	LTA4H				Mcpt1				MGL1			
M	MMP-7				Nectin-4				R-Spondin 1			
N	Serpin A6				sFRP-2				TNFb			

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 completed 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 sigmal 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. Publications Citing This Product

1. Mao Y., Yen H., Sun Y., Lv Z., Huang R. Development of non-overlapping multiplex antibody arrays for the quantitative measurement of 640 Mouse and 200 mouse proteins in parallel (TECH1P.849). The Journal of ImmunologyMay 1, 2014vol. 192 no. 1 Supplement 69.17
   Species: Mouse
   Sample Type: Serum

2. Mao Y., Yen H., Sun Y., Lv Z., Huang R. Development of non-overlapping multiplex antibody arrays for the quantitative measurement of 640 Mouse and 200 mouse proteins in parallel (TECH1P.849). The Journal of ImmunologyMay 1, 2014vol. 192 no. 1 Supplement 69.17
   Species: Mouse
   Sample Type: Plasma

More citations for this product may be available.
Contact techsupport@raybiotech.com.

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. Also,The citations listed above are for the use of this combination array. Citations for the individual arrays can be found in the individual array manuals

XIII. Experiment Record Form

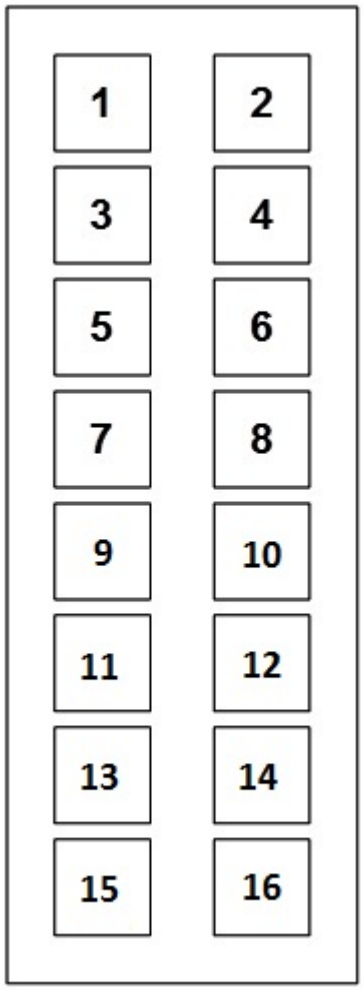
Date:_____

File Name:_____

Laser Power:_____

PMT:_____

Well No.	Sample Name	Dilution factor
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
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 660 human, 200 mouse, or 67 rat proteins. GLP-Compliant testing services are also available.

This product is for research use only.