

# G-Series Human Immune Checkpoint Molecule Array 1

Semi-quantitative measurement of 10 human immune checkpoint  
molecules

Catalog #: GSH-ICM-1

User Manual  
Last revised October 1, 2021

Caution:  
Extraordinarily useful information enclosed



ISO 13485 Certified

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**Please read the entire manual carefully before starting your experiment**

## I. Overview

<b>Cytokines Detected (10)</b>	B7-1 (CD80), B7-2 (CD86), B7-H1 (PD-L1), B7-H2 (ICOS L), B7-H3 (CD276), CD28 (Tp44), CTLA-4 (CD152), ICOS (CD278), PD-1 (CD279), PD-L2 (B7-DC) <i>See Section IX for Array Map</i>
<b>Format</b>	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
<b>Detection Method</b>	Fluorescence. Go to <a href="http://www.RayBiotech.com/Scanners">www.RayBiotech.com/Scanners</a> for a list of compatible laser scanners.
<b>Sample Volume</b>	50 - 100 µl per array
<b>Reproducibility</b>	CV <20%
<b>Assay Duration</b>	6 hours

## II. Introduction

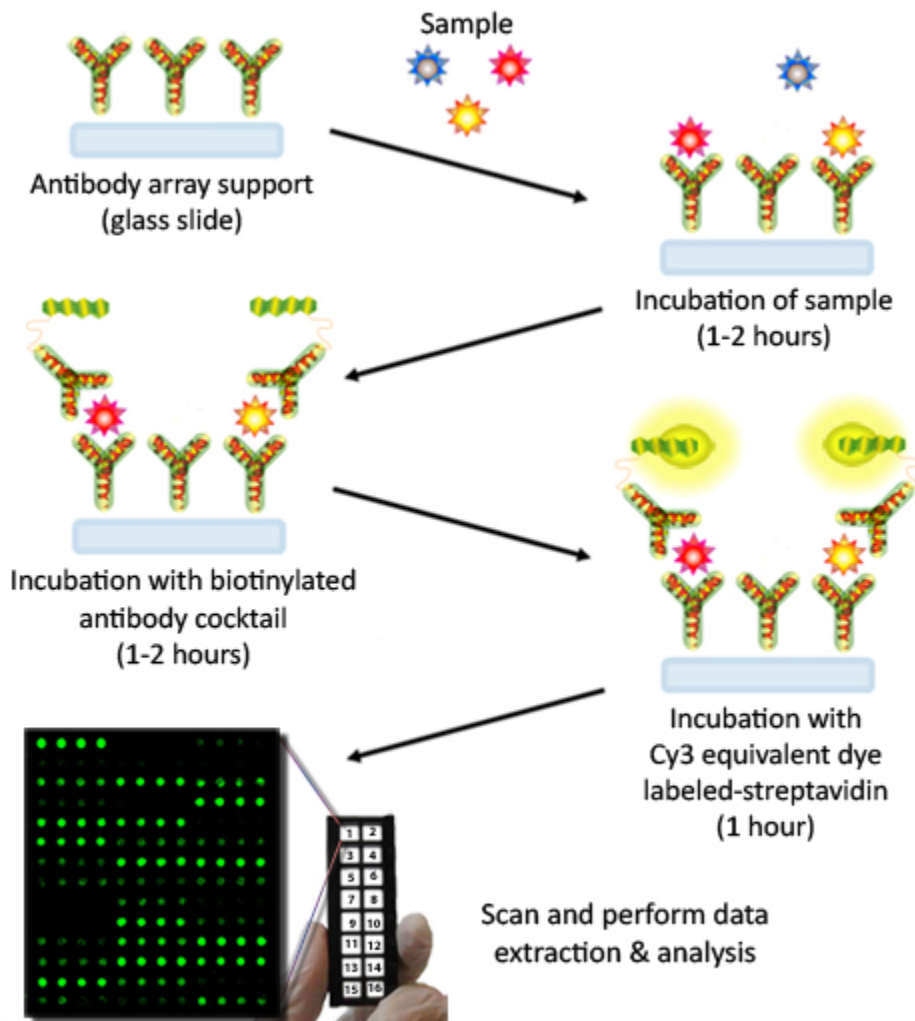
Immune checkpoint molecules play an important role in T cell functionality after TCR/MHC signaling. Blockade of two B7/CD28 family checkpoint molecules, CTLA-4 and PD-1, have already demonstrated excellent efficacy in increasing T cell responses to a variety of tumors. Recently, the United States FDA approved an anti-CTLA-4 monoclonal antibody (ipilimumab, 2011) and two anti-PD-1 monoclonal antibodies (pembrolizumab 2014, and nivolumab 2014) for the treatment of advanced melanoma. Other immune checkpoint molecules have been increasingly considered as new targets for cancer immunotherapy. In order for better identification of novel target and elucidation of the checkpoint blockade, Raybiotech is proudly offering the first human Immune Checkpoint Molecule array 1 for detection of the 10 most important B7/CD28 family checkpoint molecules: B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2/ICOSL, B7-H3, CD28, CTLA-4, ICOS, PD-1, and PD-L2/B7-DC. 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<sup>®</sup> 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  $\mu$ l per array).

### III. How It Works

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## IV. Materials Provided

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	QAH-ICM-1S	Human Immune Checkpoint Molecule Array 1 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	QAH-ICM-1B	Human Immune Checkpoint Molecule Array 1 Biotinylated Antibody Cocktail	1-25 $\mu$ l	2 x 1-25 $\mu$ l
6	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 $\mu$ l	2 x 5 $\mu$ 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

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### 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  $\mu$ 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  $\mu$ 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  $\mu$ l, or 500  $\mu$ 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<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  $\mu$ 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

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### 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  $\mu$ 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  $\mu$ 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  $\mu$ l of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500  $\mu$ g/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.*

#### 4. Wash:

- Decant the samples from each well, and wash 5 times (5 min each) with 150  $\mu$ l 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 H<sub>2</sub>O.

- *(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 H<sub>2</sub>O.

*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**

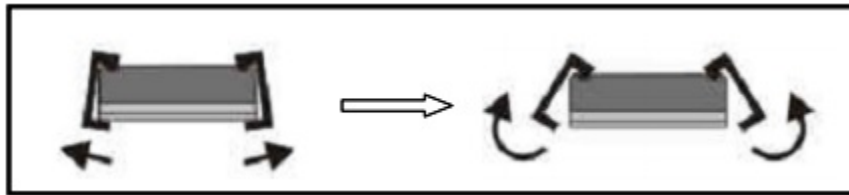
8. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.



9. Add 80  $\mu$ l of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.
10. Decant the samples from each well, and wash 5 times (5 mins each) with 150  $\mu$ l 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<sub>2</sub> stream.*

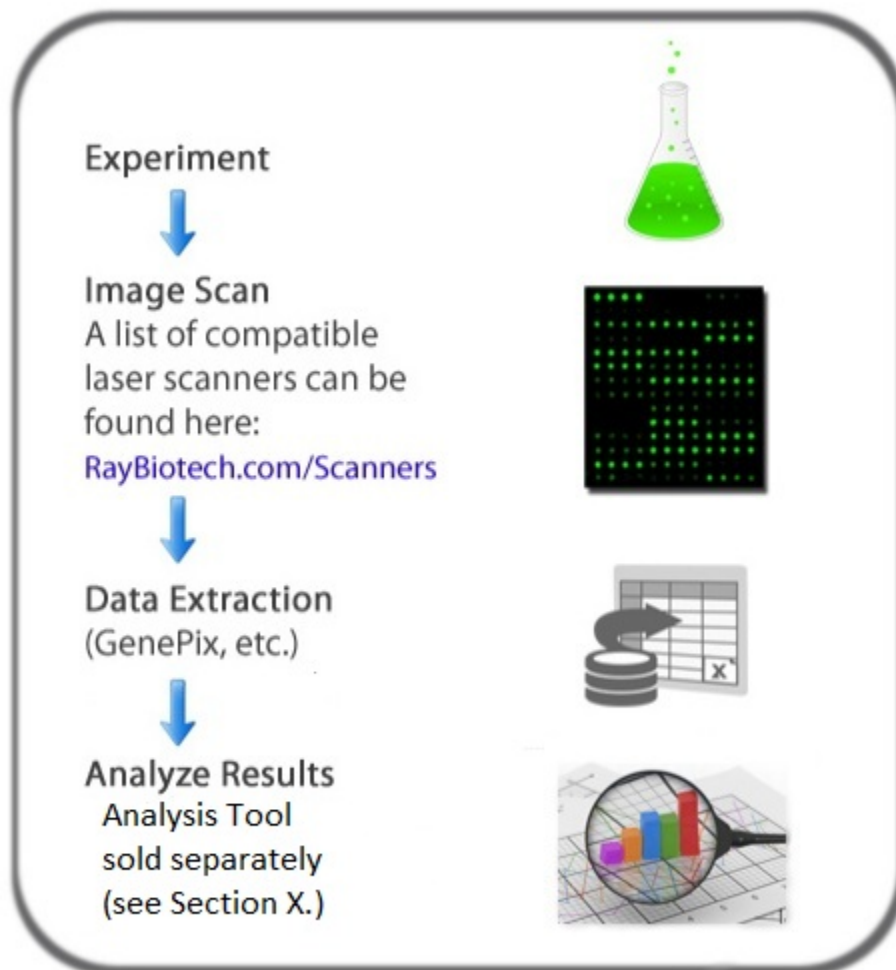
14. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan.

*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-ICM-1) 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

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Each antibody is printed in quadruplicate horizontally								
	1	2	3	4	1	2	3	4
A	<b>POS1</b>				<b>POS2</b>			
B	<b>B7-1 (CD80)</b>				<b>B7-2 (CD86)</b>			
C	<b>B7-H1 (PD-L1)</b>				<b>B7-H2 (ICOS L)</b>			
D	<b>B7-H3 (CD276)</b>				<b>CD28 (Tp44)</b>			
E	<b>CTLA-4 (CD152)</b>				<b>ICOS (CD278)</b>			
F	<b>PD-1 (CD279)</b>				<b>PD-L2 (B7-DC)</b>			

## X. Array Data Analysis Tool

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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

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
<b>Weak Signal</b>	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	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.
<b>Uneven signal</b>	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
<b>High background</b>	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

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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

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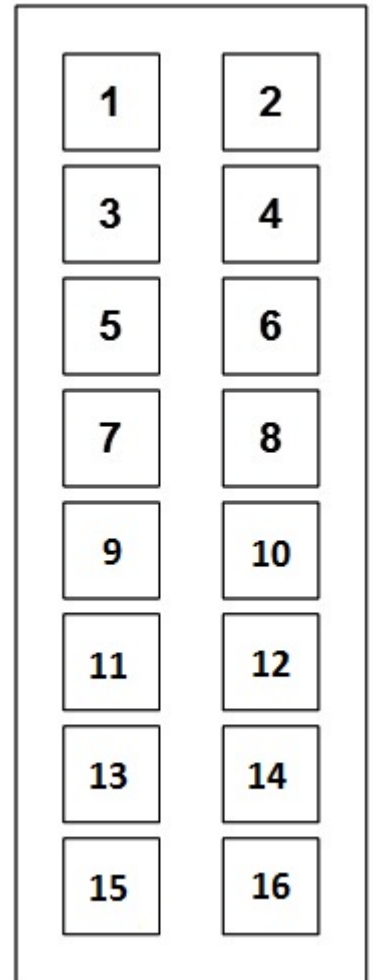
Date: \_\_\_\_\_

File Name: \_\_\_\_\_

Laser Power: \_\_\_\_\_

PMT: \_\_\_\_\_

Well No.	Sample Name	Dilution factor
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## 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	<b>Custom Arrays</b>	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<sup>®</sup> 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.



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