G-Series Human Ig Isotype Array 1

Semi-quantitative measurement of 8 human immunoglobulins

Catalog #: GSH-ISO-1

User Manual
Last revised December 5, 2019

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

ISO 13485:2016

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (8)	IgA, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM *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

The human immune system consists of two functional components classified as the innate system (the physical, biochemical and cellular barriers), and the adaptive immune system (including lymphocytes and immunoglobulins). Immunoglobulins are the key elements of the humoral immune response in vertebrate against parasitic invasion. The polypeptide chains of immunoglobulins composed of two identical heavy (H) chains and two identical light (L) chains linked together by inter-chain disulfide bonds. While the amino-terminal portions that exhibits highly variable amino-acid composition are involved in antigen binding, the C terminal constant parts are involved in complement binding, placental passage and binding to cell membranes. Based upon the variation of the constant region of the heavy chain, nine immunoglobulin heavy chain isotypes are found in humans: IgA (with subclasses IgA1 and IgA2), IgD, IgE, IgM, and IgG (with subclasses IgG1, IgG2, IgG3, and IgG4).

IgG is the predominant immunoglobulin in the serum (about 12 mg/ml), which accounts for 75% of the total serum antibody of healthy individuals. IgG has a molecular weight of about 150 kDa. Four distinct subgroups of human IgG (IgG1, IgG2, IgG3, and IgG4) were first demonstrated in the 1960s by using polyclonal antisera prepared in animals immunized with human myeloma proteins. Quantitatively, the relative abundance of the four subclasses in adult human serum follows IgG1 > IgG2 > IgG3 = IgG4, which accounts for 6.98, 3.8, 0.56, and 0.56 mg/ml respectively.

IgA exists as a 160kd monomer in serum and as a 400kd dimer in secretions. Quantitatively, IgA is synthesized in amounts greater than IgG. However, due to its short half life in serum (6 days of IgA vs 21 days of IgG) and the loss of secretory form, the normal IgA serum level (2-3 mg/ml) ranked second after IgG, which accounts for 15% of the total antibody. There are two subclasses based on different heavy chains, IgA1 and IgA2. IgA1 is produced in bone marrow and makes up over 90% of the serum IgA. Secretory IgA is the predominant immunoglobulin present in gastrointestinal fluids, nasal secretions, saliva, tears and other mucous secretions of the body.

IgM is the third most common serum immunoglobulin (about 1.5 mg/ml) which makes up about 10% serum antibody. IgM normally exists as a pentamer (about 900 kDa) and has a theoretical valence of 10. As a consequence of its pentameric structure, IgM is a good antigen agglutinating and complement fixing immunoglobulin.

IgE exists as a 190 kDa monomer and is the least common serum immunoglobulins which accounts for 0.002% of the total serum antibodies. IgE is involved in allergic reactions. If an infectious agent succeeds in penetrating the IgA barrier, it comes up against the next line of defense, the IgE manned MALT (mucosa-associated lymphoid tissues) system. Contact with the allergen leads to the release of various pharmacological mediators that result in allergic symptoms.

IgD is a 175kd molecule that resembles IgG in its monomeric form. IgD is found in low level in serum (0.03 mg/ml) with uncertain serological functions. IgD antibodies are found for the most part on the surfaces of B lymphocytes. It is thought that IgD and IgM function as mutually-interacting antigen receptors for control of B-cell activation and suppression. Hence, IgD may have an immunoregulatory function.

The levels of different immunoglobulin subclasses follow a typical pattern in a healthy ethnic adult and are normally within a certain percentile ranges. Upon different antigenic stimulation, an antibody response will behave differently in the distribution of the different subclasses in plasma, such as increase, diminish or even the deficiency of producing one of the specific immunoglobulin subclass. Over the last decades numerous reports have appeared on the distribution of the immunoglobulin subclasses produced during immune responses to bacterial, viral, and parasitic antigens; autoantigens; tumor antigens, and many parenterally administered substances such as hormones, drugs, and allergens. As a result, quantification of the immunoglobulin isotype level in a given serum sample will provide the useful information about the myeloma states as well as in monitoring intravenous immunoglobulin replacement, plasmaphoresis, and immunosuppression therapy.

Quantitative measurement of the immunoglobulin subclasses can be done with Radial Immunodiffusion assay (RID), Nephelometry and turbidimetry assay, Radio Immuno Assay (RIA), Immuno-affnity chromatography, Direct Antiglobulin Test (DAT), or Enzyme-linked Immunosorbent Assay (ELISA). While most assays can detect only one subclass of the immunoglobulin a time, taking advantage of the array technology and the availability of the

isotype specific monoclonal antibodies, Raybiotech Inc is proudly offering the research community with the Quantibody® Human Ig Isotype kit which can simultaneously and quantitatively detect multiple immunoglobulin subclasses in one experiment.

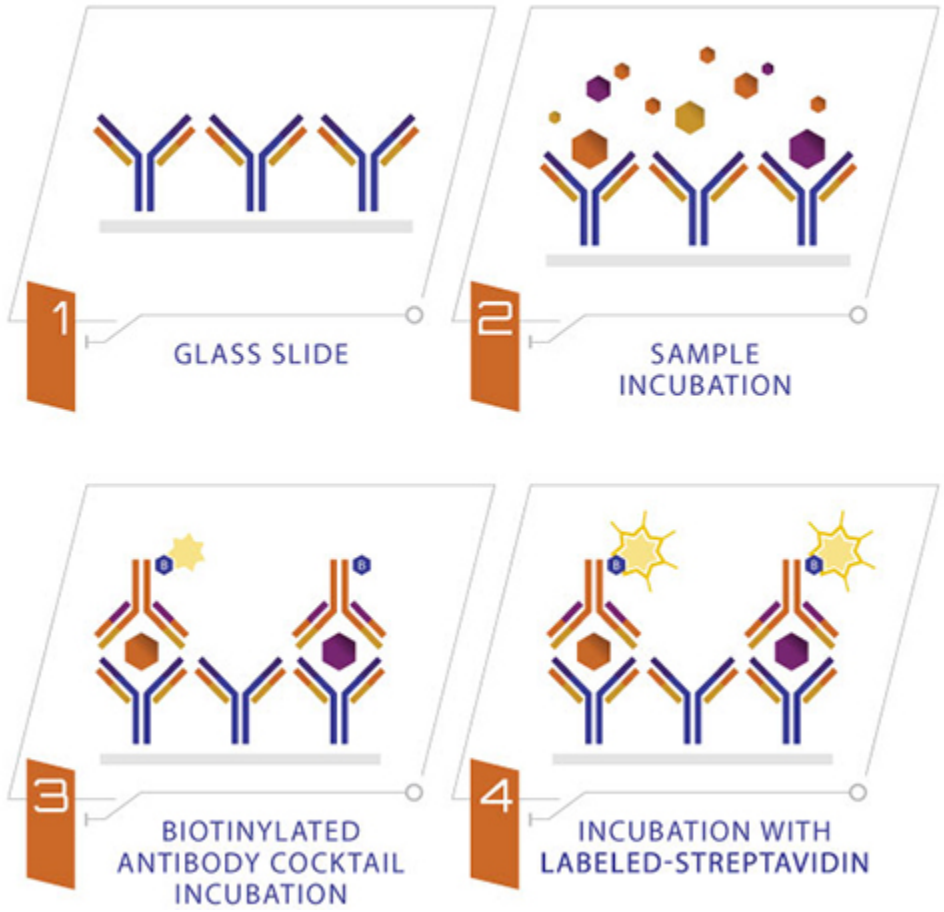
Quantibody® Human Ig Isotype Array uses sandwich-ELISA based technology for quantitative measurement of the eight human isotype immunoglobulins (IgG1, IgG2, IgG3, IgG4, IgA, IgD, IgE, and IgM) in human serum/plasma. Similar technology has been successfully used in our other Quantibody® products for quantitative measurement of up to 40 cytokines in human, mouse, rat, and porcine samples. (See Section XI). Briefly, the 8 human immunoglobulin subclass-specific antibodies are arrayed in quadruplicate (together with two positive controls) with 16 identical sub-arrays in one standard glass slide. The kit also provides a myeloma-derived standard mixture of these 8 immunoglobulins, whose concentration has been predetermined.

In the experiment, standard immunoglobulins and samples are assayed in each well simultaneously through a sandwich like ELISA procedure. The signals will be detected using fluorescence-based detection method for consistency and reliability. By comparing signals from unknown samples to the standard curve generated for each of the 8 immunoglobulins, the unknown immunoglobulin concentration in the samples will be determined.

The kit provides a highly sensitive approach (within nano gram range) to simultaneously detect 8 immunoglobulin subclasses expression levels. The experimental procedure is simple and can be performed in any laboratory. 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

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	QAH-ISO-1S	Human Ig Isotype Array 1 Glass Slide	1	2
2	QA-SDB	Sample Diluent	15 ml	2 x 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-ISO-1B	Human Ig Isotype Array 1 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 40,000x 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

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

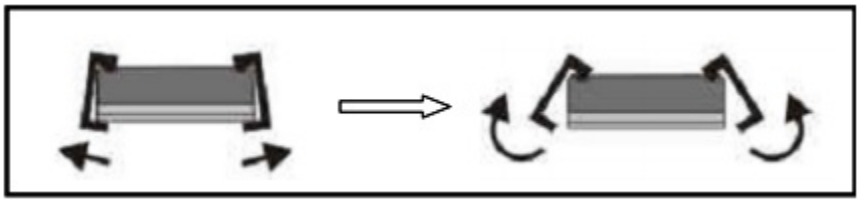
8. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

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.

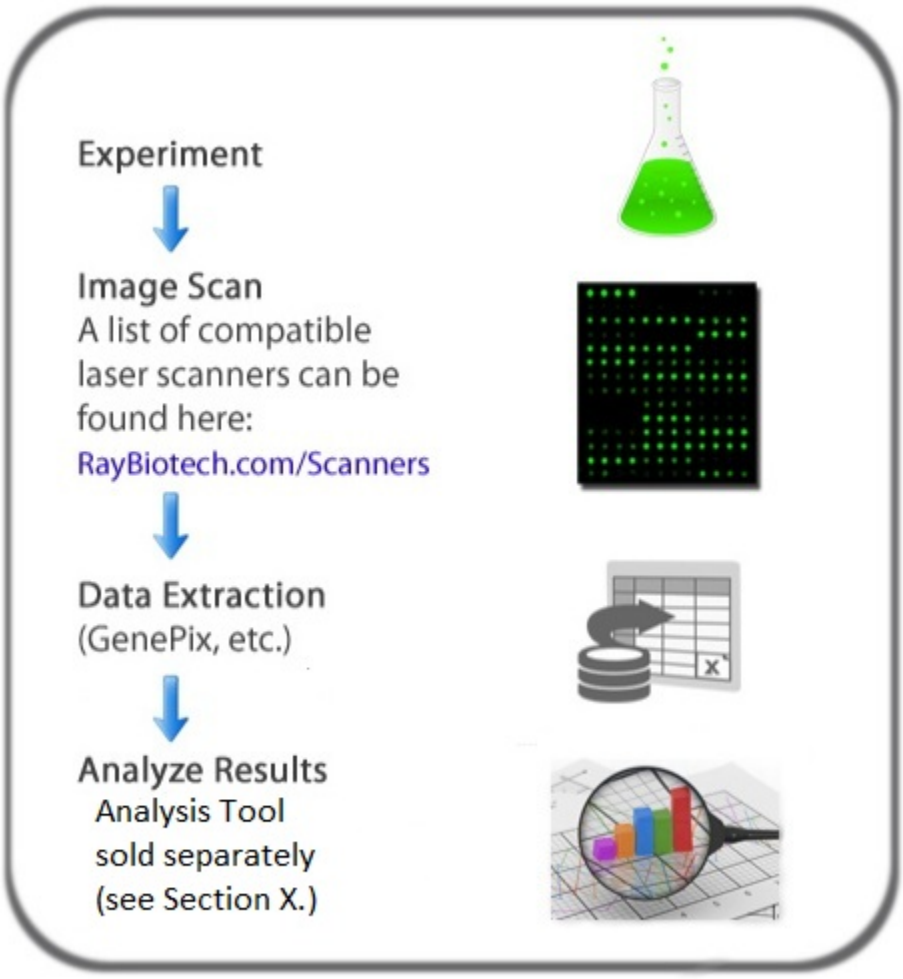
14. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan.

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 (QAH-ISO-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

Each antibody is printed in quadruplicate horizontally								
	1	2	3	4	1	2	3	4
A	POS1				POS2			
B	IgA				IgD			
C	IgE				IgM			
D	IgG1				IgG2			
E	IgG3				IgG4			

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. 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. Pharmacuetical Biology. 2009; 47(6): 500-508.
7. Cheung, et al. Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. Journal of Ethonopharmacology. 2009; 124(1): 61-68.
8. Du, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. 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 mulitplex 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

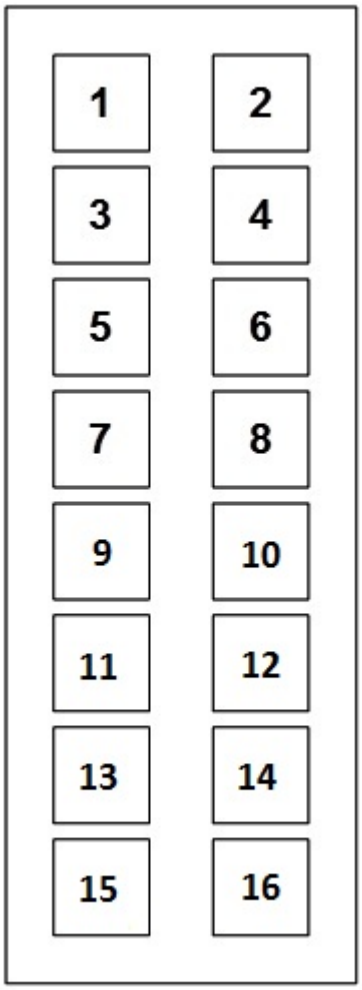
Date:_____

File Name:_____

Laser Power:_____

PMT:_____

Well No.	Sample Name	Dilution factor
1		
2		
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 1,200 human, 640 mouse, 282 rat, 50 porcine, or 30 bovine proteins proteins. GLP-Compliant testing services are also available.

This product is for research use only.

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