

# RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series

**Patent Pending Technology**

**User Manual (Revised February 18, 2009)**

RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 2 (Cat# AAM-CYT-G2-4)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 3 (Cat# AAM-CYT-G3-4)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 4 (Cat# AAM-CYT-G4-4)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 5 (Cat# AAM-CYT-G5-4)  
RayBio<sup>®</sup> Mouse Inflammation Antibody Array G Series 1 (Cat# AAM-INF-G1-4)  
RayBio<sup>®</sup> Mouse Angiogenesis Antibody Array G Series 1 (Cat# AAM-ANG-G1-4)

RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 2 (Cat# AAM-CYT-G2-8)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 3 (Cat# AAM-CYT-G3-8)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 4 (Cat# AAM-CYT-G4-8)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series 5 (Cat# AAM-CYT-G5-8)  
RayBio<sup>®</sup> Mouse Inflammation Antibody Array G Series 1 (Cat# AAM-INF-G1-8)  
RayBio<sup>®</sup> Mouse Angiogenesis Antibody Array G Series 1 (Cat# AAM-ANG-G1-8)

RayBio<sup>®</sup> Custom Mouse Cytokine Antibody Array (Cat# AAM-CUST-G)  
RayBio<sup>®</sup> Mouse Cytokine Antibody Array Service (Cat# AAM-SERV-G)

*Please read the manual carefully before you start your experiment*

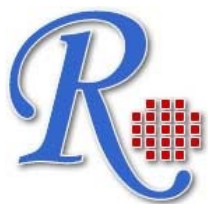


**RayBiotech, Inc.**

**We Provide You With Excellent  
Protein Array Systems and Service**

---

**Tel:(Toll Free) 1-888-494-8555 or 770-729-2992; Fax: 1-888-547-0580;  
Website:[www.raybiotech.com](http://www.raybiotech.com) Email: [info@raybiotech.com](mailto:info@raybiotech.com)**



# RayBiotech, Inc.

---

RayBio<sup>®</sup> Mouse Cytokine Antibody Array G Series Protocol

## **TABLE OF CONTENTS**

I.	Introduction.....	2
	How It Works.....	5
II.	Materials Provided.....	6
	Additional Materials Required.....	6
III.	Overview and General Considerations.....	7
	A. Preparation of Samples.....	7
	B. Handling Glass Chips .....	7
	C. Incubation.....	8
IV.	Protocol.....	9
	A. Blocking and Incubation.....	9
	B. Detection.....	10
V.	Interpretation of Results.....	13
VI.	Troubleshooting Guide.....	20
VII.	Selected References Using RayBiotech Products	21

Cytokine Antibody Arrays are RayBiotech patent-pending technology.

RayBio<sup>®</sup> is the trademark of RayBiotech, Inc.

## I. Introduction

All cell functions, including cell proliferation, cell death and differentiation, as well as maintenance of health status and development of disease, are controlled by many genes and signaling pathways. New techniques such as cDNA microarrays have enabled us to analyze the global gene expression<sup>1-3</sup>. However, almost all cell functions are executed by proteins, which cannot be studied by DNA and RNA alone. Experimental analysis clearly shows a disparity between the relative expression levels of mRNA and their corresponding proteins<sup>4</sup>. Therefore, it is critical to analyze the protein profile. Currently, two-dimensional polyacrylamide SDS page coupled with mass spectrometry is the mainstream approach to analyzing multiple protein expression levels<sup>5,6</sup>. However, the requirement of sophisticated devices and the lack of quantitative measurements greatly limit its broad application. Thus, no simple, cost effective, and rapid method of analysis of multiple protein expression levels has been available to researchers until now.

Our RayBio<sup>®</sup> Mouse Cytokine Antibody Array is the first commercially available protein array system<sup>7-11</sup>. By using the RayBiotech system, scientists can rapidly and accurately identify the expression profiles of multiple cytokines in several hours inexpensively.

The RayBiotech kit (G series) is a glass chip format. The kit provides a highly sensitive approach to simultaneously detect multiple cytokine expression levels from cell culture supernatant, patient's serum, tissue lysate and other sources. The arrays are manufactured using non-contact arrayer. The experimental procedure is simple and can be performed in any laboratory. The signals from G series arrays are detected using a laser scanner.

Besides the products listed in this manual, RayBiotech also provides RayBio<sup>®</sup> Mouse Cytokine Antibody Array G series 2000 for simultaneous detection of 144 mouse cytokines in single experiment. RayBiotech also provides RayBio<sup>®</sup> Human Cytokine Antibody Array G series 4000 which is the only product available in the market that can detect 274 human cytokines in single experiment.

Pathway-specific array systems allow investigators to focus on the specific problem and are becoming an increasingly powerful tool in cDNA microarray system. RayBiotech's first protein array system, known as RayBio<sup>®</sup> Mouse Cytokine Antibody Array, is particularly useful compared with the Mouse cytokine cDNA microarray system. Besides the ability to detect protein expression, RayBiotech's system is a more accurate reflection of active cytokine levels because it only detects secreted cytokines, and no amplification step is needed. Furthermore, it is much simpler, faster, environmentally friendlier, and more sensitive.

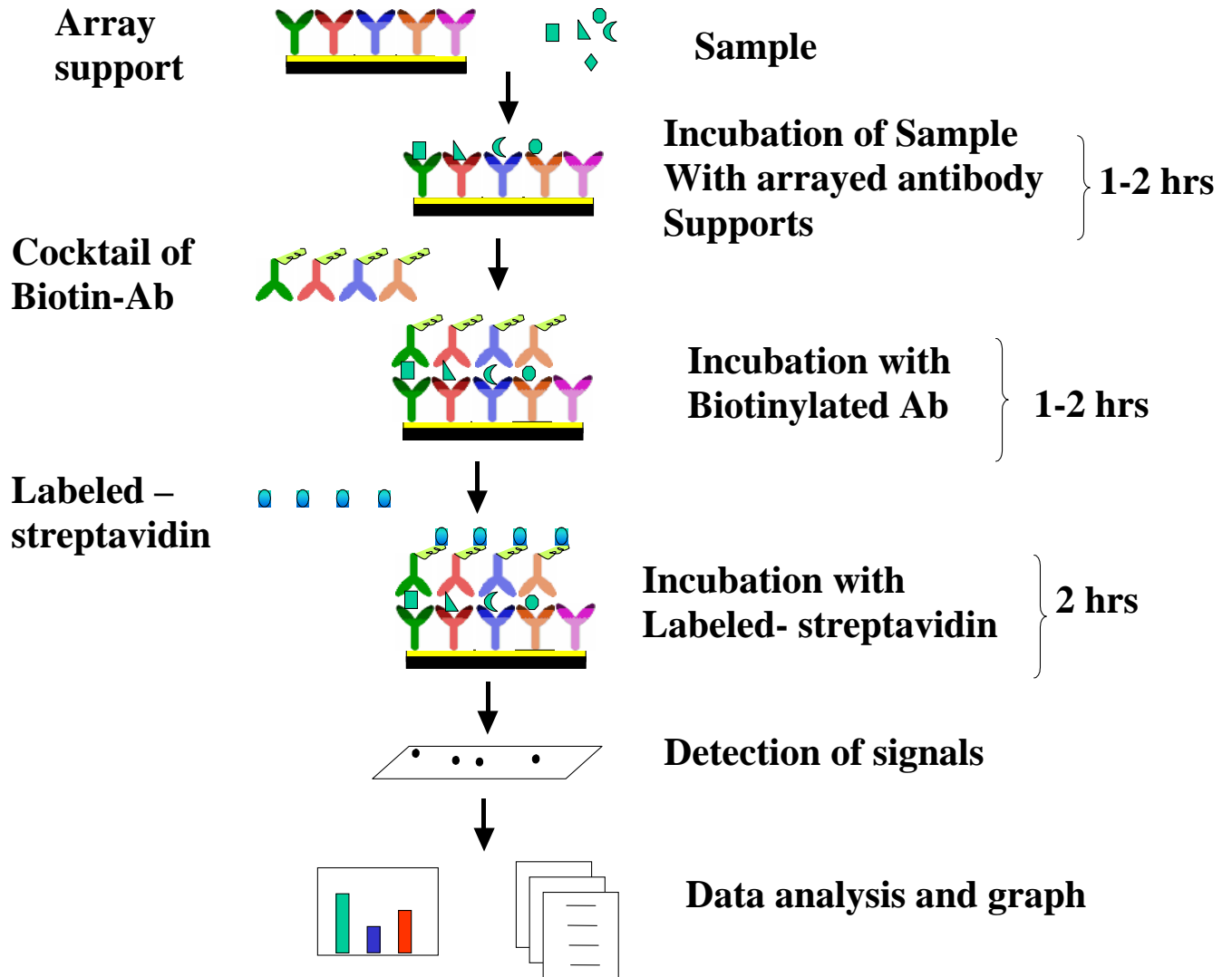
Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool to study cytokines. Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation<sup>12</sup>. Cytokines are involved in most disease processes, including cancer and cardiac diseases. The interaction between cytokines and the cellular immune system is a dynamic process. The interactions of positive and negative stimuli, and positive as well as negative regulatory loops are complex and often involve multiple cytokines.

## Reference List

1. LPS induces the interaction of a transcription factor, LPS-induced TNF- $\alpha$  factor, and STAT6(B) with effects on multiple cytokines. Xiaoren Tang, Deborah Levy Marciano, Susan E. Leeman and Salomon Amar. **PNAS**. April 5, 2005 vol. 102 no. 14 5132-5137
2. HIV-1-mediated apoptosis of neuronal cells: Proximal molecular mechanisms of HIV-1-induced encephalopathy. Yan Xu, Joseph Kulkoshy, Roger j. Pomerantz. **PNAS**. 2004 May 4, 2004 Vol. 101 No. 18.
3. Synergistic increases in intracellular Ca(2+), and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. **GLIA**. 2005 Apr 15;50(2):91-106.
4. Bone Marrow Stroma Influences Transforming Growth Factor- $\beta$  Production in Breast Cancer Cells to Regulate c-myc Activation of the Preprotachykinin-I Gene in Breast Cancer Cells. Hyun S. Oh, Anabella Moharita... Pranela Rameshwar.

5. Recombinant Herpes Simplex Virus Type 1 (HSV-1) Codelivering Interleukin-12p35 as a Molecular Adjuvant Enhances the Protective Immune Response against Ocular HSV-1 Challenge **JOURNAL OF VIROLOGY.** Mar. 2005 Vol. 79, No. 6.
6. Dysregulated Inflammatory Response to *Candida albicans* in a C5-Deficient Mouse Strain. Alaka Mullick, Miria Elias, Serge Picard, Philippe Gros. **Infection and Immunity**, Oct. 2004, p. 5868-5876, DOI: 10.1128/IAI.72.10.5868-5876.2004
7. Leukotriene B<sub>4</sub> Strongly Increases Monocyte Chemoattractant Protein-1 in Human Monocytes Li Huang, Annie Zhao, Frederick Wong, Julia M. Ayala, Jisong Cui **Arteriosclerosis, Thrombosis, and Vascular Biology.** 2004;24:1783-1788
8. Human CD1d-unrestricted NKT cells release chemokines upon Fas engagement. Martin Giroux and François Denis. Yan Xu, Joseph Kulkoshy, Roger j. Pomerantz. **Blood.** prepublished online September 2, 2004; DOI 10.1182/blood-2004-04-1537
9. Monitoring the response of orthotopic bladder tumors to granulocyte macrophage colony-stimulating factor therapy using the prostate-specific antigen gene as a reporter. Wu Q, Esuvaranathan K, Mahendran R. **Clinical Cancer Research.** 2004 Oct 15; 10(20):6977-84.
10. Neuroglial activation and neuroinflammation in the brain of patients with autism (p NA). Diana L. Vargas, Caterina Nascimbene, Chitra Krishnan, Andrew W. Zimmerman, Carlos A. Pardo. **Annals of Neurology.** 2005 Jan 1; DOI: 10.1002/ana.20315
11. Cytokine profiling of macrophages exposed to Porphyromonas gingivalis, its LPS or its FimA. Qingde Zhou, Tesfahun Desta, Dana T. Graves and Salomon Amar. **Infection and Immunity (IAI).** 2005 Feb;73(2):935-43.
12. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. Rameshwar P. **Journal of Immunology.** 2003 Oct 1;171(7):3426-34.
13. The promise of cytokine antibody arrays in drug discovery process. Huang RP, Yang W, Yang Y, Flowers L, et al. **Expert Opinion on Drug Discovery.** (2005) 9:601-615.

# Here's how it works



## II. Materials Provided

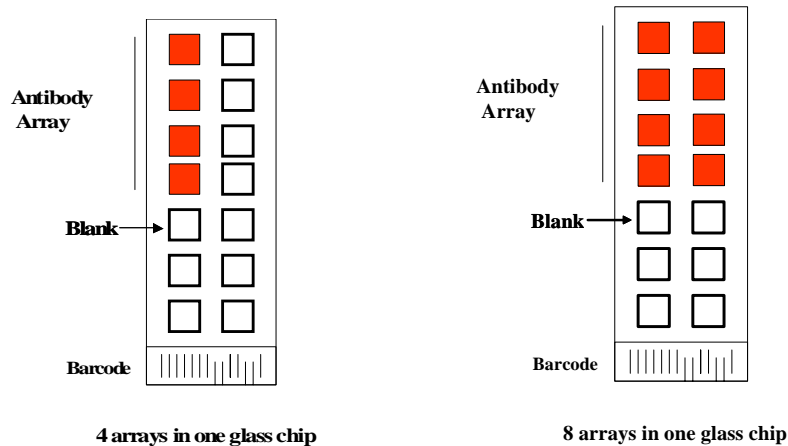
Upon receipt, all components of the RayBio<sup>®</sup> Mouse Cytokine Antibody Array kit should be stored at -20°C. At -20°C the kit will retain complete activity for up to 6 months. Once thawed, the glass chips, Fluorescent dye-streptavidin, Internal Control and 2X Blocking Buffer should be kept at -20°C and all other component should be stored at 4°C. Use within three months after reagents have been thawed. Please use within six months of purchase.

- RayBio<sup>®</sup> Mouse Cytokine Antibody Microarray slides (1 slide with 4 or 8 subarrays)
- Biotin-Conjugated Anti-Cytokines (1 tube per 4 subarrays, 1 or 2 tubes)
- 1,500X Fluorescent Dye-conjugated Streptavidin (Cy3 equivalent, 1 tube)
- 2X Blocking Buffer (10 ml)
- 20X Wash Buffer I (30 ml)
- 20X Wash Buffer II (30 ml)
- 2X Cell Lysis Buffer (10 ml)
- RayBio<sup>®</sup> G series antibody array accessory (including slide incubation chamber, Gasket, Protective cover, Snap-on sides and adhesive film)
- 30 ml tube
- Manual

## Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- Plastic box

## Layout of G series



## III. Overview and General Considerations

### A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, use uncultured media as a negative control sample, since many types of sera contain cytokines.
- For cell lysates and tissue lysates, we recommend using RayBio® Cell Lysis Buffer to extract proteins from cell or tissue (e.g. using homogenizer). Dilute 2X RayBio® Cell Lysis Buffer with H<sub>2</sub>O (we recommend adding proteinase inhibitors to Cell Lysis Buffer before use). After extraction, spin the sample down and save the supernatant for your experiment. Determine protein concentration.
- We recommend using:
  - 50–100 µl of Conditioned media (undiluted), or
  - 50–100 µl of 2-fold to 5-fold diluted sera or plasma, or
  - 10–200 µg of total protein for cell lysates and tissue lysates.

*If you experience high background, you may further dilute your sample.*

### B. Handling glass chips

- The microarray slides are sensitive, do not touch the surface. Hold the slides by the edges only.
- Handle all buffers and slides with powder-free gloves.
- Avoid breaking glass slide.
- Handle glass chip in clean environment.

### **C. Incubation**

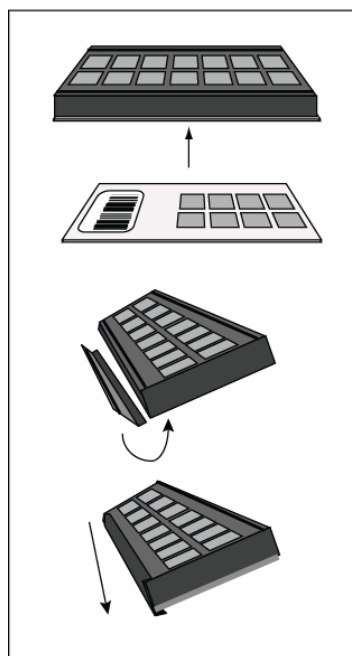
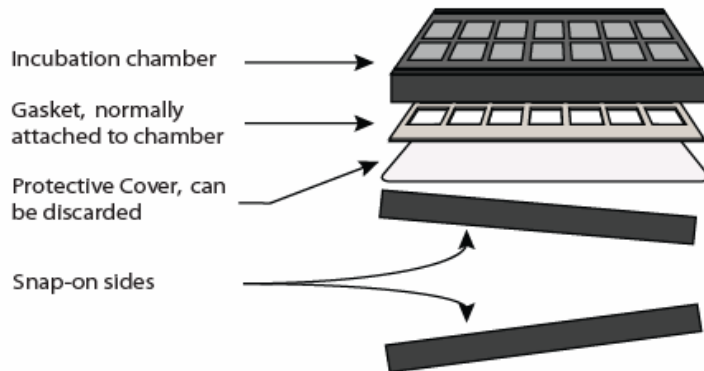
- Completely cover array area with sample or buffer during incubation, and cover the incubation chamber with adhesive film or plastic sheet protector to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or 50  $\mu$ l of sample or reagent is used.
- Avoid cross-contamination from overflowing solution to neighboring wells.
- Several incubation steps such as step 3 (blocking), step 4 (sample incubation), step 9 (biotin-Ab incubation) or step 12 (Fluorescent dye-streptavidin incubation) may be done at 4°C for overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.

## IV. Protocol

### A. Blocking and Incubation

1. Take the glass chip out from the box. Let air dry for 2 hours.
2. Assemble the glass chip into incubation chamber and incubation frame as shown below. (Note: if you slide has be assembled, you can go to step 3 directly).

#### *Instructions for incubation chamber assembly G Series and Q series arrays*



**1** Carefully place slide at bottom of the chamber as shown. The slide will adhere somewhat to the bottom. Warning: the slide is fragile, so do not apply more than gentle force to the apparatus.

**2** While gently holding chamber and slide, place side on chamber as shown, beginning with bottom flap first.

**3** Then, press the top of the side into groove on chamber, and then apply even, gentle pressure from one end to the other. Repeat this procedure with the other side.

3. Add 100  $\mu\text{l}$  1 X Blocking Buffer into each well and incubate at room temperature for 30 min to block slides. Dilute 2X Blocking Buffer with  $\text{H}_2\text{O}$ . Make sure no bubbles are in the well.

*Note: only add reagents to wells printed with antibodies.*

Decant Blocking Buffer from each well, Add 50 to 100  $\mu\text{l}$  of sample into subarray wells. Incubate arrays with sample at room temperature for 1 to 2 hours. Dilute sample using 1X Blocking Buffer if necessary.

*Note: Incubation may be done at 4°C for overnight.*

*Note: We recommend using 50 to 100  $\mu\text{l}$  of conditioned media or 50 to 100  $\mu\text{l}$  of 2-5 fold diluted serum or plasma or 10-200  $\mu\text{g}$  of protein for cell lysates and tissue lysates. **Dilute the lysate at least 10 fold with 1 X blocking buffer to make a total volume of 50 to 100  $\mu\text{l}$ . Make sure there are no bubbles in the wells.***

*Note: The amount of sample used depends on the abundance of cytokines. More concentrated sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.*

4. Decant the samples from each well, and wash 3 times with 150  $\mu\text{l}$  of 1X Wash Buffer I at room temperature with gentle shaking. 2 min per wash. Dilute 20X Wash Buffer I with  $\text{H}_2\text{O}$ . Completely remove wash buffer I in each wash step.

*Note: avoid solution flowing into neighboring wells.*

5. Put the glass chip with frame into a box with 1X Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash 2 times at room temperature with gentle shaking for 10 min per each wash.
6. Decant the 1X Wash Buffer I from each well, Put the glass chip with frame into the box with 1X Wash Buffer II (cover the whole glass slide and frame with Wash Buffer II), and wash 2 times at room temperature with gentle shaking for 5 min per each wash. Remove all of Wash Buffer II in the well. Dilute 20X Wash Buffer II with  $\text{H}_2\text{O}$ .

7. Prepare working solution for biotin-conjugated antibodies. After brief spin to ensure antibodies are at bottom of tube, add 300  $\mu$ l of 1X blocking buffer to the Biotin-Conjugated Antibody tube. Mix gently.

*Note: the diluted biotin-conjugated antibodies can be stored at 4°C for 2-3 days.*

8. Add 70  $\mu$ l of diluted biotin-conjugated antibodies to each well. Incubate at room temperature for 2 hours.

*Note: incubation may be done at 4°C for overnight.*

9. Wash as directed in steps 5 and then wash 3 times with 150  $\mu$ l of 1X Wash Buffer II at room temperature with shaking. 2 min per wash. Completely remove wash buffer II in each wash step.

10. Add 70  $\mu$ l of 1,500 fold diluted Fluorescent dye-conjugated streptavidin (after brief spin to force aliquot to bottom of tube, add 1.5 ml of Blocking Buffer to Fluorescent dye-conjugated streptavidin tube) to each subarray. Cover the incubation chamber with Adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in dark room.

11. Incubate at room temperature for 1 to 2 hours.

*Note: incubation may be done at 4°C for overnight.*

12. Wash with Wash Buffer I **twice** as directed in steps 5.

## **B. Fluorescence Detection**

1. Decant excess Wash Buffer from wells.
2. Disassemble the slide out of the incubation frame and chamber.
3. Place the whole slide in a 30 ml centrifuge tube provided, add enough Wash Buffer I (about 20 ml) to cover the whole slide and gently shake at room temperature for 10 minutes. Decant Wash Buffer I. Repeat

Wash Buffer I once. Wash with Wash Buffer II (about 20 ml) with gentle shake at room temperature for 10 minutes. Or wash using slide chamber. Rinse the slide with distilled H<sub>2</sub>O.

4. Remove water droplets by centrifuge at 1,000 rpm for 3 minutes and then let slide dry completely in air at least 20 minutes (protect from light). Make sure the slides are absolutely dry before the scanning procedure.
5. Image the signals using laser scanner such as Axon GenePix using cy3 or “green” channel (Excitation frequency = 532 nm).

*Note: we recommend scanning slides right after experiment. You also can store the slide at -20°C in dark for several days. If you do not have a laser scanner, RayBiotech can provide service for you. Just simply send your slide to us and we will take care of it.*

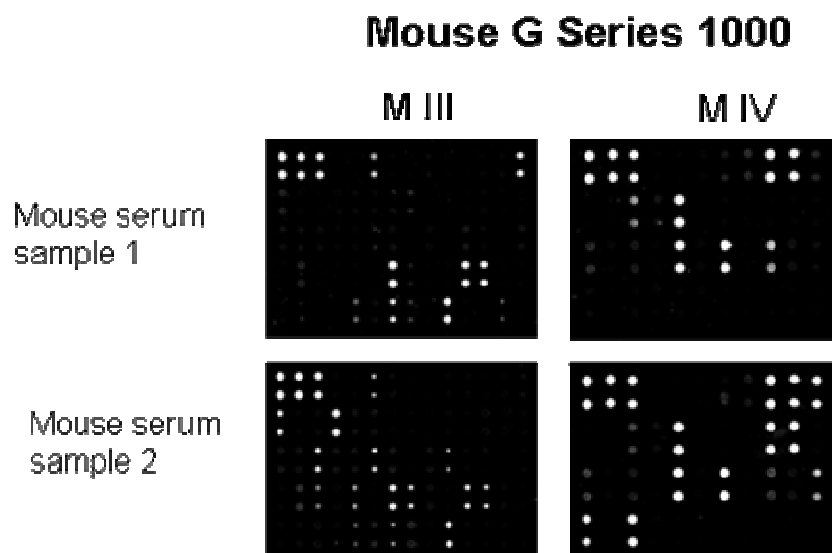
## V. Interpretation of Results:

The following figure shows RayBio<sup>®</sup> Mouse Cytokine Antibody Array G series 1000 probed with different cell culture supernatant. The images were captured using laser scanner. The biotin-conjugated protein produces positive signals, which can be used to identify the orientation and to compare the relative expression levels among the different wells. The positive signal can also be used to normalize the signal intensities among array membranes in different experiments.

The signal intensities obtained from laser scanner can simply be imported into our analysis tool. The analysis tool will help you:

- Locate your signal intensities to antibody array map
- Protein list sorting
- Average signal intensities
- Subtract background
- Normalize the data from different samples
- Obtain protein level comparison charts among different samples

This analysis tool is very simple and affordable, which will not only assist in compiling and organizing your data, but also reduces your calculations to a “copy and paste” step.



If you do not use our **RayBio<sup>®</sup> Analysis Tool**, you can locate the cytokines by referring to RayBio<sup>®</sup> Mouse cytokine Antibody Array G series 1000.

Please keep in mind that G series 1000 consists of two individual arrays; Mouse cytokine antibody array 3 and Mouse cytokine antibody array 4. Refer to corresponding map.

### Normalization and comparison

For biomarker discovery or for analysis of large number of arrays, great attention must be paid to the normalization. Our antibody array design includes several controls for normalization and comparison of arrays performing in different membranes and different experiments (for more information please read the reference 13 in the introduction).

***Positive control.*** Positive control is biotinylated protein. It can be used to normalize the streptavidin incubation step. If the positive signals from different array membranes are similar, positive control is a simple and effective way for normalization.

***Negative control.*** Negative control is BSA. Normally, it should only give a background reading.

### **Data Extraction Tips:**

- Ignore any comet tails
- Define the area for signal capture for all spots as 110-120 micron diameter, using the same area for every spot.
- Use median signal value, not the total or the mean
- Use local background correction (also median value).
- Exclude obvious outlier data in its calculations.

Using these guidelines, along with using PMT, brightness and contrast settings that reduce the background as much as possible, we get very good results with interassay and intraassay CV <20%, even with some imperfections in the antibody spots.

***Threshold of significant difference in expression:*** Any  $\geq 1.5$ -fold increase or  $\leq 0.65$ -fold decrease in signal intensity for a single analyte between samples, provided that both signals are well above background (Mean background + 2 standard deviations, accuracy  $\approx 95\%$ ).

## RayBio® Mouse Cytokine Antibody Array G series 2 (32)

	A	B	C	D	E	F	G	H	I	J
1	Pos 1	Pos 2	Pos 3	Neg	Neg	6Ckine	CTACK	Eotaxin	GCSF	GM-CSF
2	Pos 1	Pos 2	Pos 3	Neg	Neg	6Ckine	CTACK	Eotaxin	GCSF	GM-CSF
3	IL-2	IL-3	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40p70	IL-12p70	IL-13
4	IL-2	IL-3	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40p70	IL-12p70	IL-13
5	IL-17	IFN- $\gamma$	KC	Leptin	MCP-1	MCP-5	MIP-1 $\alpha$	MIP-2	MIP-3 $\beta$	RANTES
6	IL-17	IFN- $\gamma$	KC	Leptin	MCP-1	MCP-5	MIP-1 $\alpha$	MIP-2	MIP-3 $\beta$	RANTES
7	SCF	sTNFRI	TARC	TIMP-1	TNF- $\alpha$	Thrombopoietin	VEGF	Neg	Neg	Neg
8	SCF	sTNFRI	TARC	TIMP-1	TNF- $\alpha$	Thrombopoietin	VEGF	Neg	Neg	Neg

## RayBio® Mouse Cytokine Antibody Array G series 3 (62)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS 1	POS 2	POS 3	NEG	NEG	Axl	BLC	CD30 L	CD30 T	CD40	CRG-2	CTACK	CXCL16	Eotaxin
2	POS 1	POS 2	POS 3	NEG	NEG	Axl	BLC	CD30 L	CD30 T	CD40	CRG-2	CTACK	CXCL16	Eotaxin
3	Eotaxin-2	Fas Ligand	Fractalkine	GCSF	GM-CSF	IFN $\gamma$	IGFBP-3	IGFBP-5	IGFBP-6	IL-1 $\alpha$	IL-1 beta	IL-2	IL-3	IL-3 R $\beta$
4	Eotaxin-2	Fas Ligand	Fractalkine	GCSF	GM-CSF	IFN $\gamma$	IGFBP-3	IGFBP-5	IGFBP-6	IL-1 $\alpha$	IL-1 beta	IL-2	IL-3	IL-3 R $\beta$
5	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40/p70	IL-12 p70	IL-13	IL-17	KC	Leptin R	Leptin	LIX	L-Selectin
6	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40/p70	IL-12 p70	IL-13	IL-17	KC	Leptin R	Leptin	LIX	L-Selectin
7	Lymphotactin	MCP1	MCP-5	M-CSF	MIG	MIP-1 $\alpha$	MIP-1 $\gamma$	MIP-2	MIP-3 $\beta$	MIP-3 $\alpha$	PF-4	P-Selectin	RANTES	SCF
8	Lymphotactin	MCP1	MCP-5	M-CSF	MIG	MIP-1 $\alpha$	MIP-1 $\gamma$	MIP-2	MIP-3 $\beta$	MIP-3 $\alpha$	PF-4	P-Selectin	RANTES	SCF
9	SDF-1 $\alpha$	TARC	TCA-3	TECK	TIMP-1	TNF $\alpha$	sTNF RI	sTNF RII	TPO	VCAM-1	VEGF	NEG	NEG	NEG
10	SDF-1 $\alpha$	TARC	TCA-3	TECK	TIMP-1	TNF $\alpha$	sTNF RI	sTNF RII	TPO	VCAM-1	VEGF	NEG	NEG	NEG

## RayBio® Mouse Cytokine Antibody Array G series 4 (34)

	A	B	C	D	E	F	G	H	I	J	K
1	POS 1	POS 2	POS 3	NEG	NEG	bFGF	DPPIV/CD26	Dtk	E-Selectin	Fc gamma RIIB	Flt-3 Ligand
2	POS 1	POS 2	POS 3	NEG	NEG	bFGF	DPPIV/CD26	Dtk	E-Selectin	Fc gamma RIIB	Flt-3 Ligand
3	GITR	HGF R	ICAM-1	IGFBP-2	IGF-I	IGF-II	IL-15	IL-17B R	IL-7	I-TAC	Lungkine
4	GITR	HGF R	ICAM-1	IGFBP-2	IGF-I	IGF-II	IL-15	IL-17B R	IL-7	I-TAC	Lungkine
5	MDC	MMP-2	MMP-3	Osteopontin	Osteoporotegerin	Pro-MMP-9	Resistin	Shh-N	Thymus CK-1	TIMP-2	TRANCE
6	MDC	MMP-2	MMP-3	Osteopontin	Osteoporotegerin	Pro-MMP-9	Resistin	Shh-N	Thymus CK-1	TIMP-2	TRANCE
7	TROY	TSLP	VEGF R1	VEGF R2	VEGF R3	VEGF-D	NEG	NEG	NEG	NEG	NEG
8	TROY	TSLP	VEGF R1	VEGF R2	VEGF R3	VEGF-D	NEG	NEG	NEG	NEG	NEG

## RayBio® Mouse Cytokine Antibody Array 5 (48)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS 1	POS 2	POS 3	NEG	4-1BB	6Ckine	ACE /CD143	ALK-1	Amphiregulin	Cardiotrophin-1	CD27	CD27 Ligand	CD36	CD40 Ligand
2	POS 1	POS 2	POS 3	NEG	4-1BB	6Ckine	ACE /CD143	ALK-1	Amphiregulin	Cardiotrophin-1	CD27	CD27 Ligand	CD36	CD40 Ligand
3	Chordin	CTLA-4	Decorin	DKK-1	E-Cadherin	EGF	Endoglin	Epigen	Epiregulin	Galectin-1	Growth arrest specific 1	Growth arrest specific 6	GITR Ligand	Granzyme B
4	Chordin	CTLA-4	Decorin	DKK-1	E-Cadherin	EGF	Endoglin	Epigen	Epiregulin	Galectin-1	Growth arrest specific 1	Growth arrest specific 6	GITR Ligand	Granzyme B
5	HAI-1	HGF	IL-1 R4/ST2L	IL-11	IL-17B	IL-17E	IL-17F	IL-1ra/IL-1F3	IL-2 R alpha	IL-20	IL-21	IL-28	IL-6 R	JAM-A
6	HAI-1	HGF	IL-1 R4/ST2L	IL-11	IL-17B	IL-17E	IL-17F	IL-1ra/IL-1F3	IL-2 R alpha	IL-20	IL-21	IL-28	IL-6 R	JAM-A
7	MAdCAM-1	MFG-E8	Nephrilysin	Pentraxin 3	Prolactin	RAGE	TACI	TREM-1	TWEAK	TWEAK R	NEG	NEG	NEG	NEG
8	MAdCAM-1	MFG-E8	Nephrilysin	Pentraxin 3	Prolactin	RAGE	TACI	TREM-1	TWEAK	TWEAK R	NEG	NEG	NEG	NEG

## RayBio<sup>®</sup> Mouse Inflammation Antibody Array G series 1 (40)

	A	B	C	D	E	F	G	H	I	J	K	L
1	POS 1	POS 2	POS 3	NEG	NEG	BLC	CD30 L	Eotaxin	Eotaxin-2	Fas Ligand	Fractalkine	GCSF
2	POS 1	POS 2	POS 3	NEG	NEG	BLC	CD30 L	Eotaxin	Eotaxin-2	Fas Ligand	Fractalkine	GCSF
3	GM-CSF	IFN <sub>γ</sub>	IL-1 <sub>α</sub>	IL-1 β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40p70	IL-12p70
4	GM-CSF	IFN <sub>γ</sub>	IL-1 <sub>α</sub>	IL-1 β	IL-2	IL-3	IL-4	IL-6	IL-9	IL-10	IL-12p40p70	IL-12p70
5	IL-13	IL-17	I-TAC	KC	Leptin	LIX	Lymphotactin	MCP-1	MCSF	MIG	MIP-1 <sub>α</sub>	MIP-1 <sub>γ</sub>
6	IL-13	IL-17	I-TAC	KC	Leptin	LIX	Lymphotactin	MCP-1	MCSF	MIG	MIP-1 <sub>α</sub>	MIP-1 <sub>γ</sub>
7	RANTES	SDF-1	TCA-3	TECK	TIMP-1	TIMP-2	TNF <sub>α</sub>	sTNF RI	sTNF R II	NEG	NEG	NEG
8	RANTES	SDF-1	TCA-3	TECK	TIMP-1	TIMP-2	TNF <sub>α</sub>	sTNF RI	sTNF R II	NEG	NEG	NEG

## RayBio<sup>®</sup> Mouse Angiogenesis Antibody Array G series 1 (24)

	A	B	C	D	E	F	G	H
1	POS 1	POS 2	POS 3	NEG	NEG	Eotaxin	Fas Ligand	bFGF
2	POS 1	POS 2	POS 3	NEG	NEG	Eotaxin	FasLigand	bFGF
3	G-CSF	GM-CSF	IFN <sub>γ</sub>	IGF-II	IL-1 α	IL-1 β	IL-12 p40/p70	IL-12 p70
4	G-CSF	GM-CSF	IFN <sub>γ</sub>	IGF-II	IL-1 α	IL-1 β	IL-12 p40/p70	IL-12 p70
5	IL-13	IL-6	IL-9	Leptin	MCP1	M-CSF	MIG	PF-4
6	IL-13	IL-6	IL-9	Leptin	MCP1	M-CSF	MIG	PF-4
7	TIMP-1	TIMP-2	TNF α	Thrombopoietin	VEGF	NEG	NEG	NEG
8	TIMP-1	TIMP-2	TNF α	Thrombopoietin	VEGF	NEG	NEG	NEG

We also offer Custom Mouse Cytokine Antibody Arrays. You can select the cytokines of interest from the following list and we will produce the customized array at an affordable price. For more information, please visit our website, [www.raybiotech.com](http://www.raybiotech.com).

Abbreviations: IP-10, Interferon-inducible protein-10; LAP, latency associated peptide (TGF-β1); LIF, leukocyte inhibitory factor. MMP, Matrix Metalloproteinase; Pos, positive control; Neg, negative control. All other are used standard abbreviations.

Note: IL-12 reacts both IL-12p40 and IL-12p70. IL-12p70 only recognizes IL-12p70.

## RayBio® Mouse Custom Array Antibody List

Choose from 146 cytokines and other proteins

4-1BB	6Ckine	ACE	ALK-1	Amphiregulin	Axl
bFGF	BLC	Cardiotrophin-1	CD27	CD27 Ligand	CD30
CD30 Ligand	CD36	CD40	CD40 Ligand	Chordin	CRG-2
CTACK	CTLA-4	CXCL16	Decorin	DKK-1	DPPIV
Dtk	E-Cadherin	EGF	Endoglin	Eotaxin	Eotaxin-2
Epigen	Epiregulin	E-Selectin	FasLigand	Fc gamma RIIb	Flt-3 Ligand
Fractalkine	Galectin-1	Galectin-3	Galectin-7	GCSF	GITR
GITR Ligand	GM-CFS	Granzyme B	Growth arrest specific 1	Growth arrest specific 6	HAI-1
HGF	HGFR	ICAM-1	IFN gamma	IGFBP-2	IGFBP-3
IGFBP-5	IGFBP-6	IGF-I	IGF-II	IL-1 alpha	IL-1 beta
IL-1 R4/ST2L	IL-10	IL-11	IL-12 p40	IL-12 p70	IL-13
IL-15	IL-17	IL-17 B	IL-17 BR	IL-17 E	IL-17 F
IL-1ra	IL-2	IL-2 R alpha	IL-20	IL-21	IL-28/IFN-lambda
IL-3	IL-3 R beta	IL-4	IL-5	IL-6	IL-6 R
IL-7	IL-9	I-TAC	JAM-A	KC	Leptin R
LEPTIN(OB)	LIX	L-Selectin	Lungkine	Lymphotactin	MAdCAM-1
MCP1	MCP-5	M-CSF	MDC	MFG-E8	MIG
MIP-1alpha	MIP-1gamma	MIP-2	MIP-3 alpha	MIP-3 beta	MMP-2
MMP-3	Nepilysin	Osteopontin	Osteoporotegerin	Pentraxin 3/TSG-14	PF-4
Prolactin	Pro-MMP-9	P-Selectin	RAGE	RANTES	Resistin
SCF	SDF-1alpha	Shh-N	sTNF RI	sTNF RII	TACI
TARC	TCA-3	TECK	Thymus CK-1	TIMP-1	TIMP-2
TNF alpha	TPO	TRANCE	TREM-1	TROY	TSLP
TWEAK R	TWEAK	VCAM-1	VEGF	VEGF-D	VEGF R1
VEGF R2	VEGF R3				

RayBiotech, Inc., the protein array pioneer company, strives to research and develop new products to meet demands of the biomedical community. RayBio's patent-pending technology allows detection of over 180 cytokines, chemokines and other proteins in a single experiment. Our format is simple, sensitive, reliable and cost effective. Products include: Cytokine Arrays, Chemokine Arrays, ELISA kits, Phosphotyrosine kits, Recombinant Proteins, Antibodies, and custom services.

1. Antibody arrays

- Cytokine antibody array

  - Human cytokine antibody arrays

  - Mouse cytokine antibody arrays

  - Rat cytokine antibody arrays

- Pathway- or disease-focused antibody arrays

  - Inflammation antibody array

  - Angiogenesis antibody array

  - Chemokine antibody array

  - Growth factor antibody array

  - MMP antibody array

  - Atherosclerosis antibody array

- Quantibody arrays for quantitative measurement of cytokine and other protein concentration

- Phosphorylation antibody arrays

- Biotin label-based antibody arrays for high density antibody arrays.

- Antibody analysis tool, software

2. ELISA
3. Cell-based phosphorylation assay
4. Custom antibody arrays
5. Antibody
6. Recombinant protein
7. Peptide
8. Protein arrays
9. EIA

RayBiotech also provides excellent custom service:

1. Antibody arrays
2. Protein arrays
3. Peptide synthesis

4. Production of recombinant protein and antibody
5. Peptide arrays
6. Phosphorylation arrays
7. ELISA
8. EIA

Just simply send your samples and we will do the assay for you.

Technology transfer program

Have you developed technologies or reagents interested to the scientific and research community? RayBiotech can help you commercialize your technologies, reagents and dream.

## VI. Troubleshooting guide

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
Weak signal	Inadequate detection	Check laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipetters and ensure correct preparation
	Short incubation times	Ensure sufficient incubation Time and change sample incubation step to overnight
	Too low protein concentration in sample	Don't make too low dilution Or concentrate sample
	Improper storage of kit	Store kit at suggested temperature
High background	Excess of biotinylated antibodies	Make sure correct amount of antibodies
	Excess of streptavidin	Make sure correct amount of streptavidin
	Inadequate detection	Check laser power And PMT parameters
	dust	Work in clean environment
	Insufficient wash	Increase wash time and use more wash buffer
Uneven signal	Bubbles formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed Covered by reagent	Completely cover arrays with solution

## VII. Selected References Using RayBiotech Products

Transferrin-derived synthetic peptide induces highly conserved pro-inflammatory responses of macrophages. Haddad GF, Belosevic M. **Mol Immunol.** 2009; 46(4):576-586.

Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. Brochard V, Combadière B, Prigent A, Laouar Y, et al. **J Clin Invest.** 2009 ; 119 :182-192.

Human AB Serum and Thrombin-Activated Platelet-Rich Plasma Are Suitable Alternatives to Fetal Calf Serum for the Expansion of Mesenchymal Stem Cells from Adipose Tissue. Kocaoemer A, Kern S, Kluter H, Bieback K. **Stem Cells.** 2007; 25:1270-1278

Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, et al. **Nature Med.** 2007; 13(11):1359-1362.

Systemic Endocrine Instigation of Indolent Tumor Growth Requires Osteopontin. McAllister SS, Gifford AM, Greiner AL, Kelleher SP, Saelzer MP, et al. **Cell.** 2008;133:994-1005.

Chemokine Signaling via the CXCR2 Receptor Reinforces Senescence. Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, et al. **Cell.** 2008;133: 1006-1018.

Airway cytokine expression measured by means of protein array in exhaled breath condensate: Correlation with physiologic properties in asthmatic patients. Matsunaga K, Yanagisawa S, Ichikawa T, Ueshima K, et al. **J Allergy Clin Immunol.** 2006; 188:84-90.

Monocyte Chemotactic Protein-1 Mediates Prostate Cancer-Induced Bone Resorption. Lu Y, Cai Z, Xiao G, Keller ET, et al. **Cancer Res.** 2007; 67(8):3646-3653.

Proteomic Characterization of the Interstitial Fluid Perfusing the Breast Tumor Microenvironment. A Novel Resource for Biomarker and Therapeutic Target Discovery. Julio E. Celis, Pavel Gromov, Teresa Cabezón, José M. A. Moreira, Noona Ambartsumian, Kerstin Sandelin, Fritz Rank, and Irina Gromova. **Mol Cell Proteomics**. 2004; 11(3):328-39.

Antibody array-generated profiles of cytokine release from THP-1 leukemic monocytes exposed to different amphotericin B formulations. Turtinen LW, Prall DN, Bremer LA, Nauss RE, Hartsel SC. **Antimicrob Agents Chemother**. 2004;48(2):396-403.

Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 overexpression and prostaglandin e(2) secretion in patients with breast cancer. Pockaj BA, Basu GD. **Ann Surg Oncol**. 3:327-344, 2004.

Cytokine Antibody Arrays: A Promising Tool to Identify Molecular Targets for Drug Discovery. Huang, **Combinatorial Chem High Throughput Screen**. 2003, 6,79-99.

**Note:**

RayBio<sup>®</sup> is the trademark of RayBiotech, Inc.

Cytokine protein arrays are RayBiotech patent-pending technology.

This product is intended for research only and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for three months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

HiLytePlus<sup>™</sup> is a trademark of AnaSpec, Inc.



This product is for research use only.



©2009 *RayBiotech, Inc.*