RayBio® Human Acute Kidney Injury Antibody Array 1 (G Series)

Patent Pending Technology

User Manual

RayBio® Human Acute Kidney Injury Antibody Array G Series
Cat# AAH-AKI-G1-4

RayBio® Human Acute Kidney Injury Antibody Array G Series
Cat# AAH-TH17-G1-8

RayBio® Human Acute Kidney Injury Antibody Array G Series Testing Services
Cat# AAH-SERV-G

Please read manual carefully before starting experiment

RayBiotech, Inc.
the protein array pioneer company

We provide you with excellent Protein Array systems and services

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RayBiotech, Inc., the Protein Array Pioneer Company, strives to research and develop new products to meet demands of the biomedical community. RayBiotech's patent-pending technology allows detection of up to 507 cytokines, chemokines and other proteins in a single experiment. Our format is simple, sensitive, reliable, reproducible and cost-effective.

Our product offerings include:

1. Protein (antigen) Arrays
2. RayBio® Cytokine Antibody Arrays
   - C Series (Membrane, chemiluminescence detection)
   - G Series (Glass chip, fluorescence detection)
3. Pathway- and disease-focused antibody arrays
   - Angiogenesis Antibody Arrays
   - Apoptosis Antibody Arrays
   - Atherosclerosis Antibody Arrays
   - Chemokine Antibody Arrays
   - Growth Factor Antibody Arrays
   - Inflammation Antibody Arrays
   - MMP Antibody Arrays
   - Obesity Antibody Arrays
4. Quantibody® Multiplex ELISA Arrays
5. RayBio® L-Series Biotin Label-based Antibody Arrays
6. RayBio® Phosphorylation Antibody Arrays
   - Receptor Tyrosine Kinases
   - EGFR and ErbB family (site-specific phosphorylation)
7. Over 700 different ELISA kits
8. EIA (Competitive ELISA) kits
9. Cell-based Phosphorylation Assay
10. Over 10,000 different antibodies
11. Recombinant proteins
12. Peptide
13. Recombinant antibodies

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I. Introduction

New techniques such as cDNA microarrays have enabled us to analyze global gene expression[1-3]. However, almost all cell functions are executed by proteins, which cannot be studied simply through DNA and RNA techniques. Experimental analysis clearly shows disparity can exist between the relative expression levels of mRNA and their corresponding proteins[4]. Therefore, analysis of the proteomic profile is critical.

RayBiotech, The Protein Array Pioneer Company, introduced the first protein arrays to the market in 2001 and continues to lead in the development of innovative protein array technologies, such as the RayBio Human Acute Kidney Injury Antibody Array.

Acute kidney injury is a common complication among ambulatory and hospitalized patients. It is a rapidly progressive illness that independently predicts excess morbidity and mortality. It is critical to early detect acute kidney injury and distinguish it from prerenal azotemia and chronic kidney disease at the time of patient presentation to rapidly manage associated illness. However, serum creatinine, a standard marker of kidney function, does not distinguish acute kidney injury from prerenal azotemia[5] or chronic kidney disease. In addition, the initial measurement of serum creatinine cannot reflect the extent of injury because its accumulation always lags behind the insult[6].

Concurrently, the potential for improving risk stratification, informing clinical decision making, and guiding pharmaceutical development recently led the American Society of Nephrology to designate the development of novel AKI biomarkers a top research priority[7]. The response over a few years resulted in the identification of nearly 20 potential markers. Some of the more promising of these include urine or plasma Neutrophil Gelatinase-associated Lipocalin (NGAL)[8], Kidney Injury Molecule-1 (KIM-1)[9], Cystatin C[10], Liver Fatty-acid Binding Protein (L-FABP)[11], Monocyte Chemoattractant Protein 1 (MCP-1)[12] and Trefoil Factor 3 (TFF3)[13].

Traditionally, urine proteins or cytokines are detected by using ELISA. However, RayBio® Human Acute Kidney Injury Antibody Array G Series can detect 20 protein biomarkers simultaneously with small amount of sample. It is a great tool in the acute kidney injury research areas including drug toxicity monitoring, kidney transplantation rejection reaction monitoring, and kidney injury early detection.

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II. Product Information

A. Storage Recommendations:

For best results, we recommend storing the entire kit at –20°C or –80°C upon arrival and using the kit within 6 months of receipt. RayBiotech warranties this product for 6 months if stored in this manner.

Once thawed, store glass chips and 2X Blocking Buffer at –20°C or –80°C and all other component at 4°C. After thawing, the entire kit should be used within 3 months. RayBio® Antibody Array kits are robust and will retain full activity even if accidentally stored at room temperature (RT) for up to 24 hours.

B. Materials Provided

Item	Description	AAH-TH17-G1-4	AAH-TH17-G1-8
AAH-AKI1-G*X*	RayBio® Human Acute Kidney Injury Antibody Microarray Glass Chip*	1 chip with 4 Sub-arrays*	1 chip with 8 Sub-arrays*
0103002-HAK-	Biotin-Conjugated Anti-Cytokines	1 ea	2 ea
0103004-H	1,500X HiLyte Plus™ 532 Streptavidin-Fluor†	1 ea	1 ea
0103004-B	2X Blocking Buffer	10 mL	10 mL
0103004-W‡	20X Wash Buffer I ‡	30 mL	30 mL
0103004-W‡	20X Wash Buffer II ‡	30 mL	30 mL
0103004-L	2X Cell Lysis Buffer (optional)	10 mL	20 mL
Other Kit Components: Manual, Adhesive Plastic Strips, 30 mLCentrifuge Tube			

* Kit contains 1 pre-assembled glass chip with either 4 or 8 printed sub-arrays per chip (in sealed plastic envelope)
[*NOTE: In some cases, 2 chips x 4 sub-arrays/chip may be substituted in kits containing 8 sub-arrays]*

† This fluor is patent-pending technology from Anaspec, Inc.

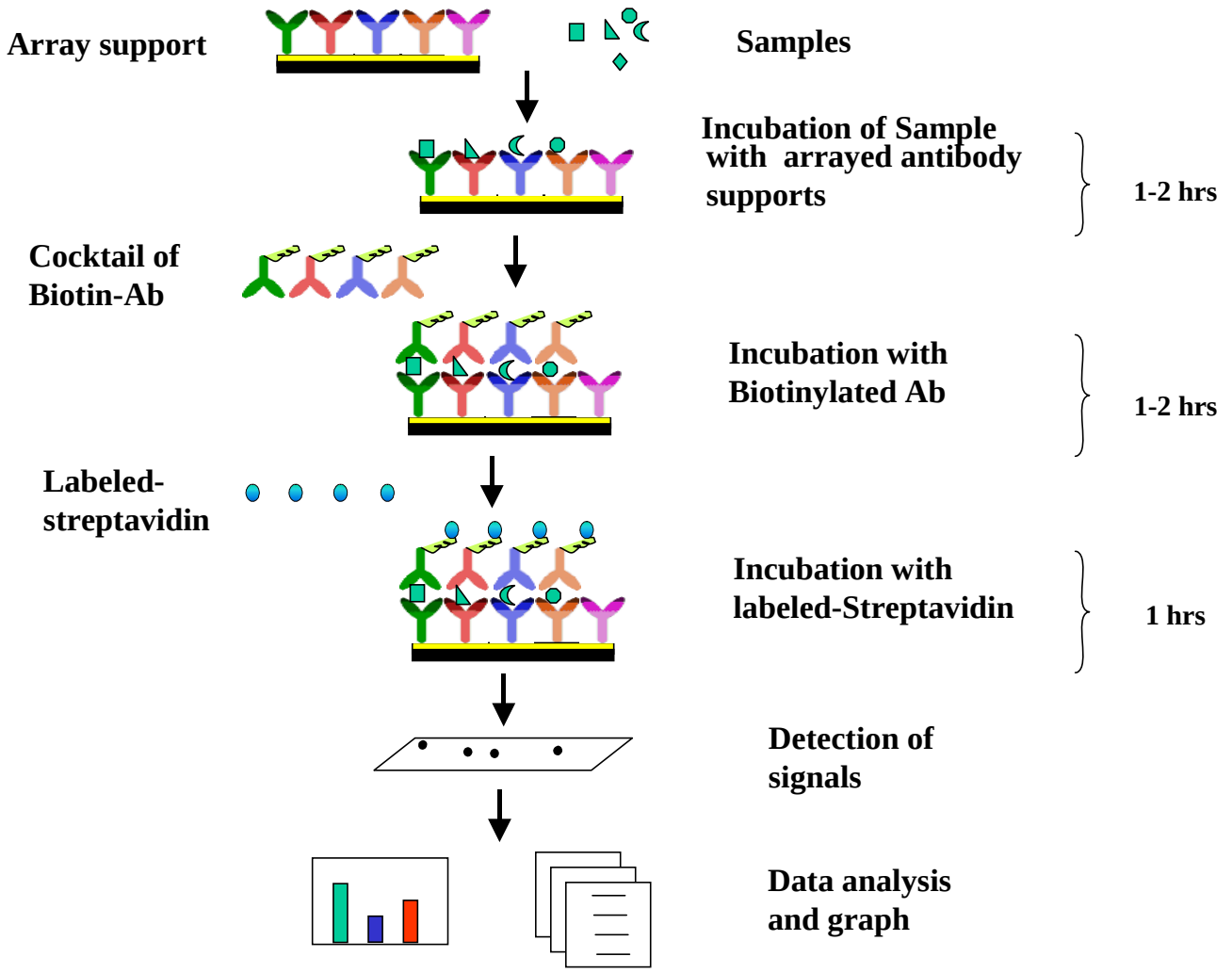
‡ Wash Buffers are sold as sets

X = 4 or 8, based on the number of printed sub-arrays on the chip

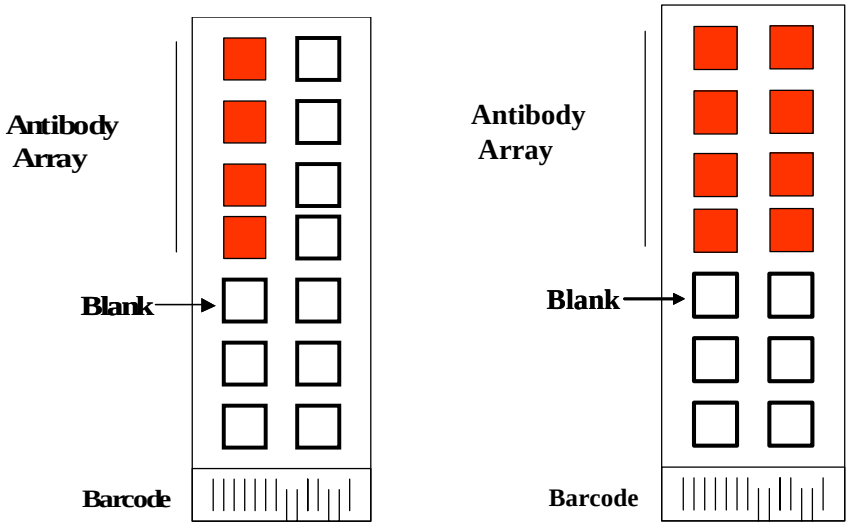
C. Additional Materials Required

- Small plastic boxes or containers
- Pipettors, pipette tips and other common lab consumables
- Orbital shaker or oscillating rocker
- Aluminum foil
- Wash bottle
- Gene microarray scanner or similar laser fluorescence scanner

D. How It Works



E. RayBio® G Series Glass Chip Layout



4 arrays in one glass chip 8 arrays in one glass chip

III. Helpful Tips and General Considerations

A. Preparation and Storage of Samples

1. General Considerations:

- Freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot your samples prior to initial storage.
- Spin samples hard (5-10 minutes at 10K to 15K RPM) immediately prior to incubation of samples with array.
- Optimal sample concentrations may need to be determined empirically based on the signal intensities of spots and background signals obtained.
- Most samples will not need to be concentrated. If concentration is required, we recommend using a spin-column concentrator with a chilled centrifuge.

2. Recommended Sample Volumes and Dilution Factors

NOTE: All sample dilutions should be made using 1X Blocking Buffer. For all sample types, final sample volume = 50-100 µL per sub-array

- **Urine** 2-fold to 5-fold dilution

Note: The RayBio® Acute Kidney Injury Antibody Array is intended for use with human urine samples. However, if you wish, you may test other sample types as follows:

- ***Serum & Plasma**: 2-fold to 5-fold dilution*

3. Preparing Urine:
   - Prepare 500 µL aliquots and store at –20°C or –80°C as soon as possible after collecting urine samples.
   - Addition of protease inhibitors is not required.
   - Immediately prior to sample incubation (Step 3 of protocol), spin samples at 1000 rpm for 10 minutes to remove particulates and precipitants.

4. Preparing Serum/Plasma:
   - Prepare samples according to established protocols or collection tube manufacturer's instructions. Sub-aliquot into plastic tubes. Store at –20°C or –80°C.
   - We do not recommend comparing results between serum and plasma samples or between plasma prepared using different anticoagulants.
   - You may test plasma samples prepared using any anticoagulant (i.e., Heparin, EDTA or Citrate). However, EDTA-prepared plasma may interfere with optimal detection of MMPs and other metal-binding proteins.
   - If possible, avoid testing hemolyzed serum or plasma, as these samples may generate anomalous cytokine expression patterns and/or high background signals.

B. Handling Glass Chips
   - Do not remove glass chip from assembly until Step 16.
   - Hold the slides by edges only; do not touch the surface.
   - Handle all buffers and slides with powder-free gloves.
   - Dry glass chip completely before proceeding to Step 3.
   - Handle and dry glass chip in clean environment.

- Avoid breaking glass chip when removing the chamber assembly.

C. Incubations and Washes

- Cover incubation chamber with adhesive film (included in kit) to prevent evaporation, particularly during incubation or wash steps >2 h or with liquid volumes <100 µL per well.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/s).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 4°C.
- Overnight sample incubations are the most effective at increasing sample spot intensities.
- Avoid cross-contamination of samples to neighboring wells
- To remove Wash Buffers and other reagents from chamber wells, you may invert the Incubation Chamber Assembly to decant, and aspirate the remaining liquid.
- In Wash Steps 6, 12 and 15, you may gently flush wells several times using a wash bottle filled with Wash Buffer I.

D. Scanning and Data Extraction Tips:

For tips on scanning and data extraction, please visit our Website: http://www.raybiotech.com/Tech-Support/ScanningTips.pdf

For a list of recommended scanners, please visit our Website: http://www.raybiotech.com/resources.asp.

IV. Protocol

A. Preparation and Storage of Reagents

NOTE: During this protocol, prepare reagents immediately prior to use and keep working dilutions of all reagents on ice at all times.

1. Blocking Buffer (Item# 0103004-B) is supplied as 2X.
   a). For glass chips with 4 sub-arrays each, prepare at least 2.5 ml (1.25 mL 2X Blocking Buffer + 1.25 mL deionized H_20).

b). For glass chips with 8 sub-arrays each, prepare at least 3.0 ml (1.5 mL 2X Blocking Buffer + 1.5 mL deionized H_20).
c). If your samples require dilution prior to incubation with the sub-arrays, increase this volume accordingly.
d). Store 1X and 2X Blocking Buffer at –20°C or –80°C when not in use.

2. Wash Buffers I and II (Item# 0103004-W) are supplied as 20X.
   a). For each glass chip (4 or 8 sub-arrays/chip), dilute 5 mL of 20X concentrate with deionized H_20 to a final volume of 100 mL each of Wash Buffer I & Wash Buffer II.
   b). Wash Buffer reagents at 1X can be stored at 4°C for up to 1 month. Stock solutions at 20X can be stored 4°C for up to 3 months.

3. Biotin-conjugated Anti-Cytokines are supplied as a small liquid bead (typically ~2-5 µL) of highly concentrated antibodies.
   a). Spin down the tube prior to reconstitution, as the concentrated liquid bead may have moved to the top of the tube during handling.
   b). Prepare stock reagent by adding 300 µL 1X Blocking Buffer to Biotin-Conjugated Anti-Cytokines. Mix well.
   c). 1X Biotin-Conjugated Anti-Cytokines may be stored for 2-3 days at 4°C.

4. Streptavidin-Fluor is supplied as 1500X.
   a). Mix the tube containing 1500X Streptavidin-Fluor well before use, as precipitants may form during storage.
   b). Add 100 µL of 1X Blocking Buffer to tube containing 1500X Streptavidin-Fluor. Mix well.
   c). Quantitatively transfer all of Streptavidin-Fluor reagent from the original tube to a larger one, and dilute with 1X Blocking Buffer to a final volume of 1500 µL (i.e., 1.5 mL).
   d). This working dilution can be stored for 3-5 days at 4°C.

B. Blocking and Incubations

NOTE: Please carefully read Section III of this manual before proceeding

NOTE: Prepare all reagents immediately prior to use as described above (Section IV.A) before proceeding.

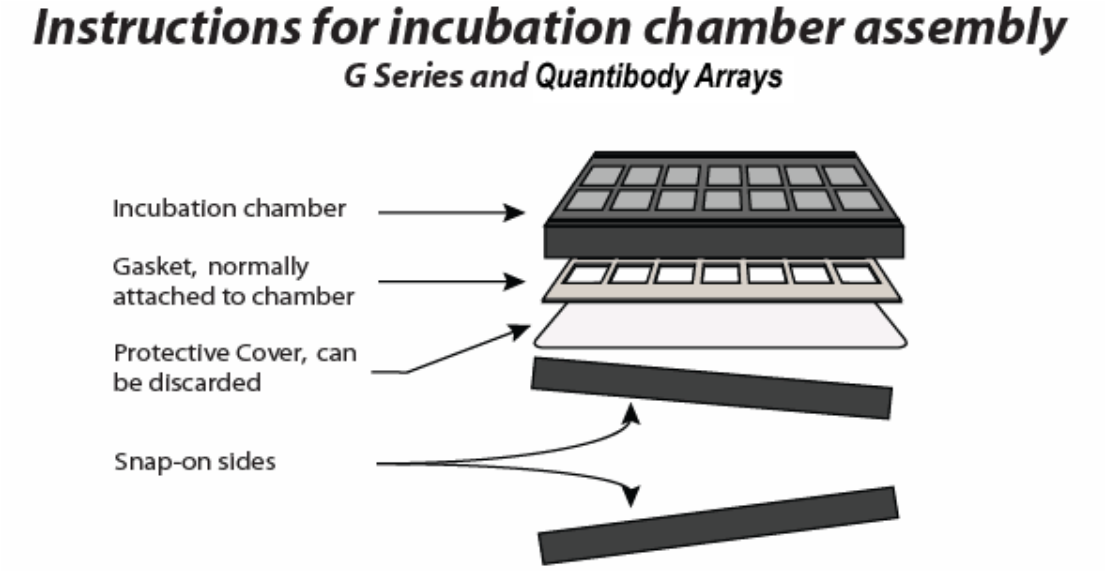
1) Remove the package containing the Glass Chip Assembly from the freezer. Place unopened package on the benchtop and allow the it to equilibrate to room temperature (RT), approx. 15 min. Open package, remove the glass chip assembly and place in laminar flow hood to dry for 1-2 hours.

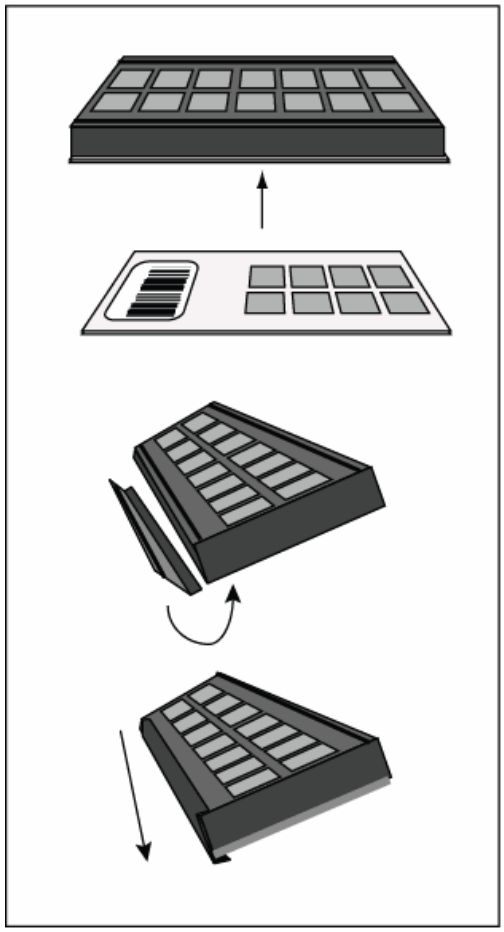
NOTE: Be sure glass chip is completely dry before proceeding.

2) If necessary, assemble the glass chip into Incubation Chamber and frame as shown on pages 12-13. (Note: if you slide is already assembled, you can proceed directly to Step 3).

3) Add 100 µL 1 X Blocking Buffer into each well and incubate at RT for 30 min to block array surface.

NOTE: Only add reagents or samples to wells printed with antibodies (see diagram on page 7)





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 grove on chamber, and then apply even, gentle pressure from one end to the other. Repeat this procedure with the other side.

4) Decant Blocking Buffer; then aspirate remaining liquid.

NOTE: To aspirate liquid samples or reagents from wells, gently place the pipette tip only in the corners of the well. Do not scrape the pipette tip across the surface of the chip.

5) Add 50 to 100 µL of each sample to each sub-array. Cover the incubation chamber with Adhesive Film (included in kit). Incubate arrays with sample at RT for 1 to 2 hours with gentle rocking or shaking. Dilute sample using 1X Blocking Buffer if necessary.

6) Remove adhesive film, and carefully aspirate samples from sub-arrays, touching only the corners with your pipette tip.

7) Wash each array 3 times, 2 min per wash with 150 µL 1X Wash Buffer I at RT. Be sure to completely remove sample and Wash Buffer each time and use fresh buffer for each wash. Decant final wash solution before proceeding to next step.

NOTE: Try to prevent solution from flowing into neighboring wells.

8) Obtain a clean container (e.g., pipette tip box or slide staining jar) and place Glass Chip Assembly into the container. Add enough 1X Wash Buffer I to submerge the entire glass chip with frame intact (approx. 30-50 ml) and remove all bubbles in wells. Wash 10 min at RT with gentle rocking or shaking.

9) Remove Glass Chip Assembly and invert it to decant liquid. Decant buffer from container and replenish with 1X Wash Buffer. Again submerge the entire glass chip assembly, and wash 10 min at RT with gentle rocking or shaking.

10) Remove Glass Chip Assembly and invert to decant liquid. Decant buffer from container and repeat Steps 8 & 9 with 1X Wash Buffer II.

11) Invert Glass Chip Assembly to decant liquid, then carefully aspirate wash buffer from wells, touching only the corners with your pipette tip.

12) Add 70 µL of 1X Biotin-conjugated Anti-Cytokines to each sub-array. Cover Incubation Chamber with Adhesive Film. Incubate at RT for 2 hours with gentle rocking or shaking.

13) Carefully aspirate Biotin-conjugated Anti-Cytokine reagent. Wash as described in Step 7 above, first with 1X Wash Buffer I then with 1X Wash Buffer II, making sure to completely remove buffer between washes and after final wash.

14) Add 70 µL of 1X Streptavidin-Fluor to each sub-array. Cover the incubation chamber with Adhesive film, then cover entire assembly with aluminum foil to avoid exposure to light or

incubate in dark room. Incubate at RT for 2 hours with gentle rocking or shaking.

15) Remove aluminum foil and Adhesive Film. Carefully aspirate Streptavidin-Fluor reagent. Wash as described in Step 7 above, first with 1X Wash Buffer I then with 1X Wash Buffer II, making sure to completely remove buffer between washes and after final wash.

16) Remove glass chip from the frame assembly. Place the chip in 30 mL Centrifuge Tube (provided in kit), or slide staining jar. Add enough 1X Wash Buffer I to cover the whole slide (about 20 mL) and gently rock or shake at RT for 10 min.

17) Decant buffer and repeat wash as described in Step 16, this time using 1X Wash Buffer II. Decant buffer, remove the glass chip from the tube, then gently rinse the slide with distilled H_2O using a plastic wash bottle.

18) Remove excess liquid from 30 mL Centrifuge Tube and place glass chip into the tube. Centrifuge at 1,000 RPM for 3 minutes to remove water droplets.

C. Obtaining Fluorescent Signal Intensities:

19) Remove chip from tube and allow glass chip to dry in a laminar flow hood for at least 20 minutes. Place chip under an aluminum foil tent to protect it from light. Make sure the slides are completely dry before scanning or storage.

20) You may proceed immediately to scanning (Step 21), or you may store the slide at -20 °C in the centrifuge tube provided or at RT and to scan at a later time.

Note: Unlike most Cy3 fluors, the HiLyte Plus™ Fluor 532 used in this kit is very stable at RT and resistant to photobleaching on completed glass chips. However, please protect glass chips from strong light and temperatures above RT.

21) Scan the glass chip with a laser scanner (such as Axon GenePix) using Cy3 or "green" channel (excitation frequency = 532 nm). For tips on scanning, visit our Website: http://www.raybiotech.com/Tech-Support/ScanningTips.pdf

NOTE: If you do not have a laser scanner, RayBiotech offers scanning and data extraction services for a nominal fee. Also, using alternate protocols, RayBio® G Series arrays are compatible with Li-Cor's Odyssey and Gentel BioScience's APiX scanners. For more information, contact RayBiotech.

V. Interpretation of Results:

A. Explanation of Controls Spots

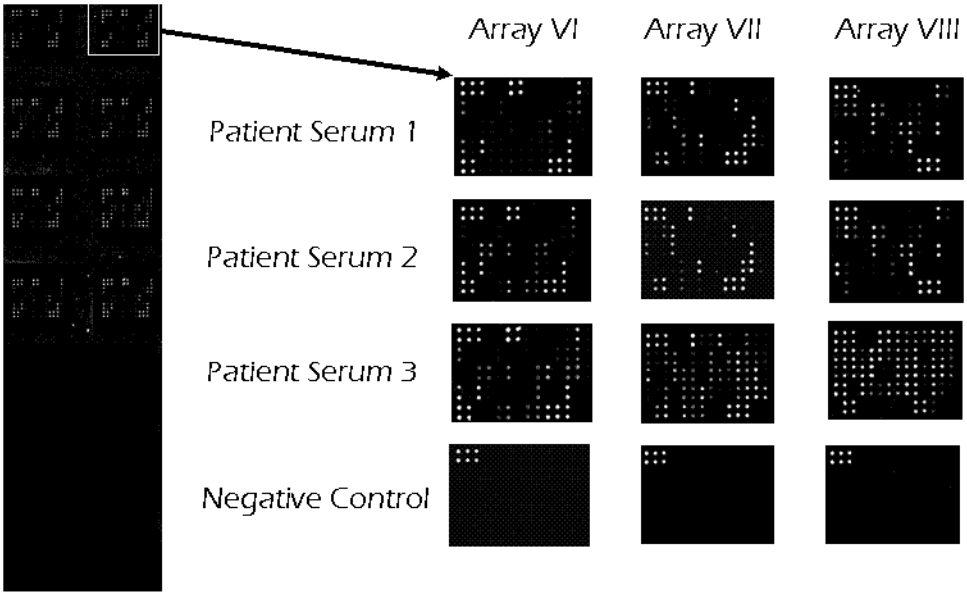
Positive Controls (POS1, POS2, POS3) are equal amounts of biotinylated IgGs printed directly onto the array. All other variables being equal, the Positive Control intensities will be the same for each sub-array This allows for normalization based upon the relative fluorescence signal responses to a known control, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

Negative Control (NEG) spots are a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of Biotin-conjugated anti-Cytokines and/or Steptavidin-Fluor. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical results obtained with RayBio® G Series Antibody Arrays

The following figure shows typical results obtained using RayBio® G Series Antibody Arrays. The images were captured using a GenePix 4000B scanner.

Sera from several patients were incubated with Human Cytokine Arrays 6, 7 & 8, (sold together as Human Cytokine Array G Series 2000, AAH-CYT-G2000-4 or AAH-CTY-G2000-8) and processed using this standard protocol.



Note the 6 strong signals of the Positive Control spots in the upper-left corner. These spots are useful for proper orientation of the array image.

If scanned using optimal scan settings, 3 distinct Positive Control signal intensities will be seen: POS1>POS2>POS3. If all of these signals are of similar intensity, try increasing or decreasing laser power and/or signal gain settings.

Once you have obtained fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

C. Background Subtraction:

Most laser fluorescence scanner software have an option to automatically measure the local background around each spot. As

with spot signal intensities, we recommend using MEDIAN background signals. If your resulting fluorescence signal intensity reports do not include these values (eg, a column labeled as "MED532-B532"), you may need to subtract the background manually or change the default settings on your scanner's data report menu.

D. Normalization of Array Data:

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice can be arbitrary. For example, in our Analysis Tool Software, the array represented by data entered in the left-most column each worksheet is the default "reference array."

You can calculate the normalized values as follows:

$$X(Ny) = X(y) * P1/P(y)$$

Where:

P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny)= normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is available for use with data obtained using RayBio® G Series Arrays. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls.

To order the Analysis Tool, please contact us at +1-770-729-2992 or info@raybiotech.com for more information.

E. Threshold of significant difference in expression:

After subtracting background signals and normalization to Positive controls, comparison of signal intensities for antigen-specific antibody spots between and among array images can be used to determine relative differences in expression levels of each analyte (ie, protein detected) between samples or groups.

Any ≥1.5-fold increase or ≤0.65-fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy ≈ 95%).

NOTE: In the absence of an external standard curve for each analyte, there is no means of assessing absolute or relative concentrations of different analytes in the same sample using immunoassays. If you wish to obtain quantitative data (ie, concentrations of the various analytes in your samples), try using our Quantibody® Multiplex ELISA arrays instead.

VI. RayBio® Human Acute Kidney Injury Antibody Array G Series Map:

Detects 20 cytokines in one experiment

	A	B	C	D	E	F	G	H
1	POS	POS	NEG	NEG	KIM-1	ALB	OPN	TFF3
2	POS	POS	NEG	NEG	KIM-1	ALB	OPN	TFF3
3	B2M	Clusterin	CXCL16	GPNMB	L-FABP	MCP-1	sTNFRI	Calbindin-1
4	B2M	Clusterin	CXCL16	GPNMB	L-FABP	MCP-1	sTNFRI	Calbindin-1
5	IP-10	Cystatin C	HGF	MIF	NGAL	TIMP-1	VCAM-1	VEGF
6	IP-10	Cystatin C	HGF	MIF	NGAL	TIMP-1	VCAM-1	VEGF
7	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS
8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS

Abbreviations:

POS = Positive Control, NEG = Negative Control, KIM-1 = Kidney Injury Molecule-1, ALB = Albumin, B2M = β2-Microglobulin, OPN = Osteopontin, L-FABP = Liver Fatty-Acid Binding Protein, HGF = Hepatocyte Growth Factor, MIF = Macrophage migration Inhibitory Factor, NGAL= Neutrophil Gelatinase-Associated Lipocalin (Lipocalin 2). All others use standard abbreviations.

VII. Troubleshooting guide

Problem	Cause	Recommendation
No signal for any spots, including Positive Controls	Global detection failure	Adjust scanner settings or re-assemble chip into holder, wash slide 2 x 5 min with 150 µL Wash Buffer II and repeat Steps 12-19.
Similar signal intensities for POS1/2/3	Improper laser power and/or PMT setting	Repeat scan using higher and/or lower laser power or PMT settings
High background signals	Incomplete washes	Carefully follow wash protocols, and/or increase wash times
	Sample concentration is too high	Repeat using lower sample concentration
	Fluor and/or Anti-Cytokines are too concentrated	Review protocol for dilution of reagents
Uneven background and/or missing spots	Bubbles present on chip during incubations	Be sure to completely remove all bubbles from chip surface
	Evaporation during incubation steps	Cover chamber assembly during washes and incubations
	Pooling/precipitation of sample or reagent; Incomplete washes.	Cover chamber assembly and use a rocker or shaker during washes and incubations; carefully follow wash protocols.
	Sample is too concentrated	Repeat experiment using more dilute sample
Randomly scattered high-intensity spots	Dust or other particulates	Dry slides in laminar flow hood and/or use clean containers and powder-free gloves.

Weak or no signals antigen-specific spots + Low Background	Sample is too dilute	Repeat experiment using higher sample concentration
	Improper dilution of Anti-Cytokines or Streptavidin-Fluor	Re-assemble chip into holder, wash 2 x 5 min with 150 µL Wash Buffer II and repeat Steps 12-19. Spin down reagents before diluting and mix well.
	Other Tips	Rescan at higher laser power or signal gain setting
		Repeat using higher sample concentration and/or incubate with sample O/N at 4°C
		Increase concentration of and/or length of incubation with Biotin-conjugated Anti-Cytokine (+ addl'l large volume wash following Biotin-Ab incubation
		Review proper storage conditions for kit components

III. Selected References Citing RayBio® Human G-Series Arrays

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4-1BB	CNTF	GITR	IL-18 BPα	MIP-1δ	SAA
ACE-2	Cripto	GITR Ligand	IL-18 Rβ	MIP-3α	sgp130
Acrp30	CRP	GM-CSF	IL-1ra	MIP-3β	Shh N
Activin A	CTACK	GRO	IL-2	MMP-1	Siglec-5
Adiposin	CXCL16	GROα	IL-2 Rβ	MMP-10	Siglec-9
Adipsin	DAN	GH	IL-2 Rγ	MMP-13	ST2
AgRP	Decorin	HB-EGF	IL-2 Ra	MMP-2	sTNF RI
ALCAM	Dkk-1	HCC-4	IL-21R	MMP-3	sTNF RII
α-Fetoprotein	Dkk-3	hCG (intact)	IL-22	MMP-7	TACE
Amphiregulin	Dkk-4	HGF	IL-28A	MMP-8	TARC
Angiogenin	DPPIV	HVEM	IL29	MMP-9	TECK
Angiopoietin-1	DR6	I-309	IL-3	MPIF-1	TGFα
Angiopoietin-2	Dtk	ICAM-1	IL-31	MSPα	TGFβ1
Angiostatin	E-Cadherin	ICAM-2	IL-4	NAP-2	TGFβ2
ANGPTL4	EDA-A2	ICAM-3	IL-5	NCAM-1	TGFβ3
Axl	EGF	IFNγ	IL-5 Rα	NGF R	TPO
B7-1	EGFR	IGF-1 SR	IL-6	Nidogen-1	Thyroglobulin
BCAM	EG-VEGF	IGFBG-1	IL-6 sR	NrCAM	Tie-1
BCMA	ENA-78	IGFBP-2	IL-7	NRG1-β1	Tie-2
BDNF	Endoglin	IGFBP-3	IL-8	NT-3	TIM-1
β2M	Eotaxin	IGFBP-4	IL-9	NT-4	TIMP-1
β IG-H3	Eotaxin-2	IGFBP-6	Insulin	Oncostatin M	TIMP-2
bFGF	Eotaxin-3	IGF-I	IP-10	Osteopontin	TIMP-4
BLC	Ep CAM	IGF-I SR	I-TAC	OPG	TNFα
BMP-4	ErbB2	IGF-II	LAP	PAI-I	TNFβ
BMP-5	ErbB3	IL-1α	Leptin	PARC	TNFRSF21
BMP-6	EPO R	IL-1β	Leptin R	PDGF Rα	TNFRSF6
BMP-7	E-Selectin	IL-1 R II	LIF	PDGF Rβ	TRAIL R2
β-NGF	Fas	IL-1 R4/ST2	LIGHT	PDGF-AA	TRAIL R3
BTC	Fas Ligand	IL-1 RI	LIMPII	PDGF-AB	TRAIL R4
CA125	Fcr RIIB/C	IL-1 sRI	L-Selectin	PDGF-BB	Trappin-2
CA15-3	Ferritin	IL-10	LH	PECAM-1	TREM-1
CA19-9	FGF-4	IL-10 Rα	Lymphotactin	PIGF	TSH
CA IX	FGF-6	IL-10 Rβ	LYVE-1	PF4	TSLP
Cardiotrophin-1	FGF-6	IL-11	Marapsin	Procalcitonin	Ubiquitin
Cathepsin S	FGF-7	IL-12	MCP-1	Prolactin	uPAR
CCL14a	FGF-9	IL-12 p40	MCP-2	PSA-free	VCAM-1
CCL21	Fit-3 Ligand	IL-12 p70	MCP-3	PSA-total	VE-Cadherin
CCL-28	FLRG	IL-13	MCP-4	RAGE	VEGF
CD14	Follistatin	IL-13 Rα-2	M-CSF	RANK	VEGF R2
CD23	Fractalkine	IL-13 RI	M-CSF R	RANTES	VEGF R3
CD30	FSH	IL-15	MDC	Resistin	VEGF-C
CD40	Furin	IL-16	MICA	S-100b	VEGF-D
CD40 Ligand	Galectin-7	IL-17	MICB	SAA	XEDAR
CD80	GCP-2	IL-17B	MIF	SCF	
CEA	G-CSF	IL-17C	MIG	SCF R	
CEACAM-1	GDF-15	IL-17F	MIP-1α	SDF-1	
CK b 8-1	GDNF	IL-17R	MIP-1β	SDF-1β	

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