

# RayBio<sup>®</sup> Rat Acute Kidney Injury Antibody Array 1 (C-Series)

Patent Pending Technology

## User Manual

RayBio<sup>®</sup> Rat Acute Kidney Injury Antibody Array C-Series  
Cat# AAR-AKI-1-4

RayBio<sup>®</sup> Rat Acute Kidney Injury Antibody Array C-Series  
Cat# AAR-AKI-1-8

RayBio<sup>®</sup> Rat Cytokine Antibody Array Service  
Cat# AAR-SERV

Please read manual carefully  
before starting experiment



We provide you with excellent Protein Array systems and services

---

Tel: (Toll Free) 1-888-494-8555 or +1-770-729-2992; Fax: +1-770-206-2393;  
Website: [www.raybiotech.com](http://www.raybiotech.com) Email: [info@raybiotech.com](mailto:info@raybiotech.com)

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 1000 cytokines, chemokines and other proteins in a single experiment. Our format is simple, sensitive, reliable and cost effective.

Our product offerings include:

1. Protein (antigen) Arrays
2. Cytokine Antibody Arrays (Human, Mouse, Rat and Porcine)
  - 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. L-Series Biotin Label-based Antibody Arrays
6. Phosphorylation Antibody Arrays
  - Receptor Tyrosine Kinases
  - EGFR and ErbB family (site-specific phosphorylation)
7. Over 700 different ELISA kits
8. EIA kits
9. Cell-based phosphorylation assay
10. Over 10,000 different Antibodies
11. Recombinant proteins
12. Peptide
13. Recombinant antibodies



# Protocol for RayBio<sup>®</sup> Rat Acute Kidney Injury Antibody Array 1 (C Series)

---

## TABLE OF CONTENTS

I.	Introduction.....	1
II.	Product Information.....	3
	A. Storage Recommendations.....	3
	B. Materials Provided.....	4
	C. Additional Materials Required.....	4
	D. How It Works.....	5
III.	Helpful Tips and General Considerations.....	5
	A. Preparation and Storage of Samples.....	5
	B. Handling Array Membranes.....	7
	C. Incubations and Washes.....	7
	D. Chemiluminescence Detection.....	8
IV.	Protocol.....	8
	A. Preparation and Storage of Reagent.....	8
	B. Blocking and Incubations.....	9
	C. Chemiluminescence Detection.....	10
V.	Interpretation of Results.....	12
VI.	Antibody Array Maps.....	15
VII.	Troubleshooting Guide.....	16
VIII.	Selected References.....	17

RayBio<sup>®</sup> Cytokine Antibody Arrays are patent-pending technology.  
RayBio<sup>®</sup> is the trademark of RayBiotech, Inc.

## I. Introduction

New techniques such as cDNA microarrays have enabled us to analyze global gene expression<sup>1-3</sup>. 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<sup>4</sup>. 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 Rat 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<sup>5</sup> 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<sup>6</sup>.

The kidney is the primary organ responsible for the excretion of medications and their biotransformation products from the body. Rats are widely used for probing pharmacokinetic-pharmacodynamic (PK-PD) relationships for medications; in addition, rats have been demonstrated to be a useful model for evaluating mechanisms of kidney toxicity. In recent years, numerous molecules have been described and investigated as candidate biomarkers of kidney injury. The United States Food and Drug Administration (FDA) has taken an active role in developing a process for qualification of biomarkers<sup>7, 8</sup> that would potentially improve the drug development and regulatory review process. In the gentamicin-induced rat model of acute kidney

injury, based on histopathology, necrosis, or apoptosis scoring, kidney injury molecule-1 (KIM-1) was the best biomarker of overall renal injury<sup>9</sup>.

Traditionally, urine proteins or cytokines are detected by using ELISA. However, RayBio<sup>®</sup> Rat Acute Kidney Injury Antibody Array C-Series can detect 7 protein biomarkers simultaneously with a small amount of sample. It is a great tool in the acute kidney injury research areas and drug discovery area to hasten drug development.

1. Tang X, Marciano DL, Leeman SE, Amar S. LPS induces the interaction of a transcription factor, LPS-induced TNF- $\alpha$  factor, and STAT6(B) with effects on multiple cytokines. *PNAS*. 2005; 102(14): 5132-5137.
2. Xu Y, Kulkosky J, Acheampong E, Nunnari G, Sullivan J, Pomerantz RJ. HIV-1-mediated apoptosis of neuronal cells: Proximal molecular mechanisms of HIV-1-induced encephalopathy. *PNAS*. 2004; 101(18): 7070-7075.
3. El-Hage N, Gurwell JA, Singh IN, Knapp PE, Nath A, Hauser KF. 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; 50(2):91-106.
4. Oh HS, Moharita A, Potian JG, Whitehead IP, et al. 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. *Cancer Res*. 2004; 64: 6327-6336.
5. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. *J Am Soc Nephrol*. 2003; 14:2199-210.
6. Lameire N, Hoste E. Reflections on the definition, classification, and diagnostic evaluation of acute renal failure [Editorial]. *Curr Opin Crit Care*. 2004; 10:468-75.
7. Goodsaid F, Frueh F. Biomarker qualification pilot process at the US Food and Drug Administration. *AAPS J* 2007; 9: E105-E108.
8. Goodsaid FM, Frueh FW, Mattes W. Strategic paths for biomarker qualification. *Toxicology* 2008; 245: 219-223.

9. Rodney L. Rouse, Jun Zhang, Sharron R. Stewart, Barry A. Rosenzweig, Parvaneh Espandiari, and Nakissa K. Sadrieh<sup>3</sup>. Comparative profile of commercially available urinary biomarkers in preclinical drug-induced kidney injury and recovery in rats. *Kidney International* (2011) 79, 1186-1197

## II. Product Information

### A. Storage Recommendations:

For best results, store the entire kit at -20°C or -80°C upon arrival. If stored frozen, we recommend using the kit within 6 months, which is the duration of the product warranty period.

Once thawed, store array membranes and 1X 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. Array kits are robust and will retain full activity even if stored for up to 24 hours at room temperature.

## B. Materials Provided

Item	Description	AAR-AKI-1-4	AAR-AKI-1-8
AAR-AKI1- <i>Y</i>	RayBio® Rat AKI Antibody Array Membranes*	1 or 2 paks*	2 paks*
0103002- R-AKI -1	Biotin-Conjugated Anti-Cytokines	2 ea	4 ea
0103004-H	1,000X HRP-Conjugated Streptavidin	1 ea	1 ea
0103004-B	1X Blocking Buffer	25 ml	50 ml
0103004-W †	20X Wash Buffer I †	10 ml	20 ml
0103004-W †	20X Wash Buffer II †	10 ml	20 ml
0103004-D †	Detection Buffer C †	1.5 ml	2.5 ml
0103004-D †	Detection Buffer D †	1.5 ml	2.5 ml
0103004-T	8-Well Plastic Tray	1	1
<b>Other Kit Components:</b>			
8-well Tray, Plastic sheets, Manual, Array Template, Packing list			

\* Packs contains 2 or 4 arrays each

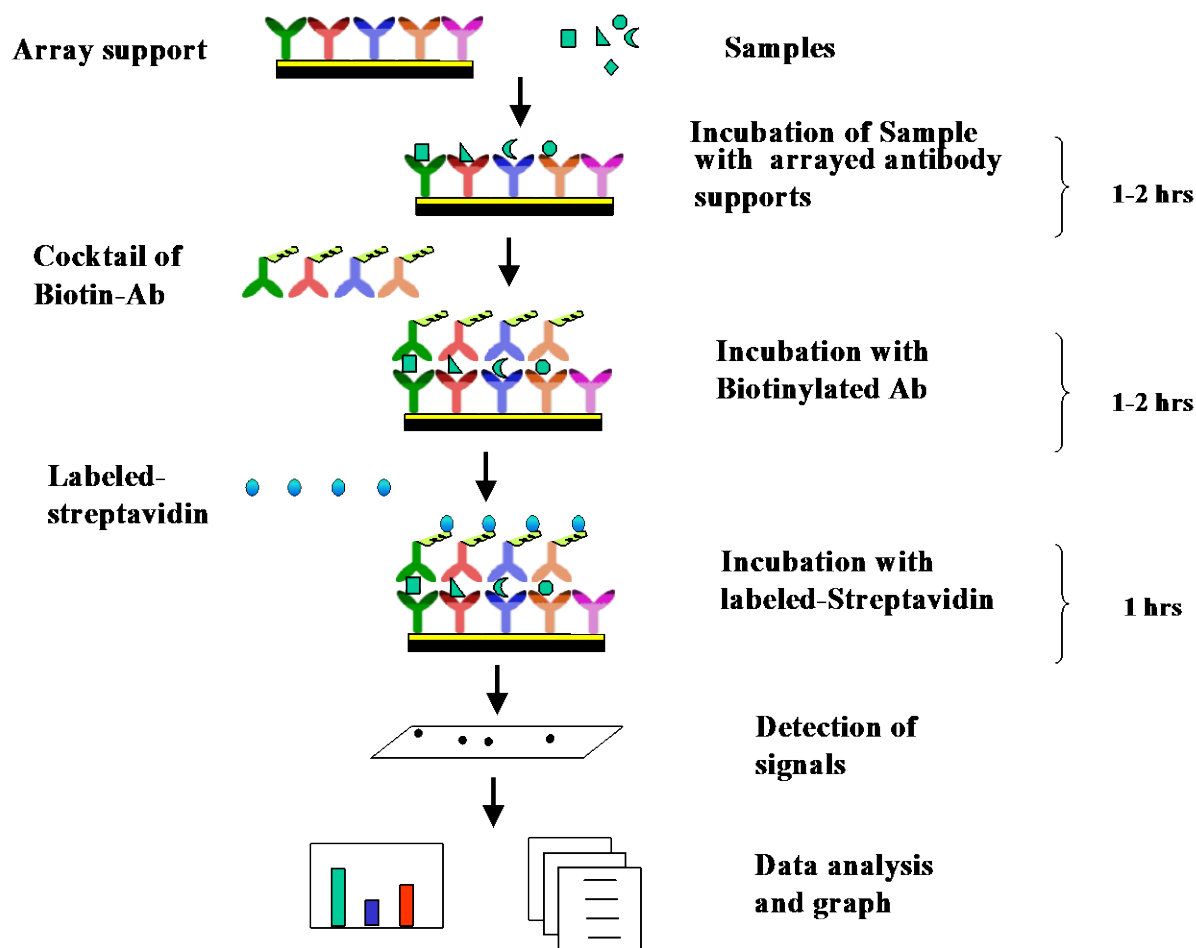
† Wash Buffers and Detection Buffers are sold as Sets

*Y* = 4 or 8

## C. Additional Materials Required

- Small plastic boxes or containers
- Pipettors, pipet tips and other common lab consumables
- Orbital shaker or oscillating rocker
- Saran Wrap or similar plastic film
- A chemiluminescent blot documentation system (such as UVP's ChemiDoc-It® or EpiChem II Benchtop Darkroom), X-ray Film and a suitable film processor, or other chemiluminescent detection system.

## D. How It Works



## 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 with each sample.
- If spot intensities are weak, increase sample concentration in subsequent experiments.
- If background or spot intensities are too strong, decrease sample concentration in subsequent experiments.



- 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 the 1X Blocking Buffer provided in this kit. For all sample types, final sample volume = 1.0-1.2 mL per membrane***

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

***Note:*** If the sample volume is less than 200µl, the membrane and sample may be sealed in a small plastic bag to increase sample-membrane coverage. Expel all air bubbles prior to sealing bag.

***Note:*** The RayBio<sup>®</sup> Rat Acute Kidney Injury Antibody Array is intended for use with Rat 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 200µl 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 precipitates.

## 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.

- For most applications, you may test plasma samples prepared using any anticoagulant (i.e., Heparin, EDTA or Citrate). However, EDTA-prepared plasma may interfere with 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 Array Membranes

- Array membranes are fragile when dry. Handle with care.
- Wet or dry, grasp membranes by the edges using forceps.
- Do not allow membranes to dry out during experiments.
- The printed side of each membrane is denoted by a dash mark (-) or array number in the upper left corner.

#### C. Incubations and Washes

- All washes and incubations in the standard protocol can be performed using the 8-well tray provided in the kit.
- Place the cover on 8-well trays with lid to avoid drying, particularly during extended incubation or wash steps.
- During each incubation, be sure to completely cover the membranes with sample or reagent.
- During incubation steps, avoid foaming and be sure to remove all bubbles from the membrane surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion ( $\approx 0.5$  to 1 cycles/second).
- 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 antigen-specific spot intensities.
- If you perform overnight sample incubations, we recommend adding the optional “Large Volume Wash” described in Step 4 to minimize background signals.
- Overnight blocking and wash steps are useful for reducing background signal intensities. Wash steps may be repeated even with completed membranes to reduce background signals. Wash with Wash Buffer II, followed by repeating incubation with Streptavidin-HRP and chemiluminescent

detection may greatly improve signal-to-noise ratios in your developed array images.

#### D. Chemiluminescence Detection

- We strongly recommend using multiple exposures to obtain optimum images. Begin by exposing the membranes for 40 seconds. Then re-expose the film accordingly.
- If the signals are too strong (or background is too high), reduce exposure time (e.g., 5-30 seconds).
- If the signals are weak, increase exposure time.
- Gel/blot documentation systems that use CCD cameras to detect chemiluminescence are ideal for imaging RayBio<sup>®</sup> array membranes. They can easily be programmed to take multiple exposures, and the dynamic range of these detectors tends to be 2-3 orders of magnitude greater than X-ray film.

### 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 is supplied as 1X concentration, no reconstitution or dilution is required. Store 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 membrane, dilute 1 mL of Wash Buffer I with deionized water to a final volume of 20 mL.
  - b). For each membrane, dilute 1 mL of Wash Buffer II with deionized water to a final volume of 20 mL.
  - c). 1X Wash Buffers can be stored at 4°C for up to 1 month. 20X Wash Buffers can be stored 4°C for up to 3 months.
3. Biotin-Conjugated Anti-Cytokines are supplied at 2000X concentration as a small liquid bead (typically ≈2-5 µL).

*Note: Spin down the tube prior to reconstitution, as the concentrated liquid bead may have moved to the top of the tube during handling.*

- a). Add 100  $\mu$ L 1X Blocking Buffer to the tube containing 2000X Biotin-Conjugated Anti-Cytokines.
  - b). Mix well and quantitatively transfer stock reagent to larger tube containing 1900  $\mu$ L of 1X Blocking Buffer.
  - c). 1X Biotin-Conjugated Anti-Cytokines may be stored for 2-3 days at 4°C.
4. Streptavidin-HRP is supplied as 1000X concentration.
- a). Mix the tube containing 1,000X Streptavidin-HRP well before use, as precipitates may form during storage.
  - b). Add 2  $\mu$ L of 1000X Streptavidin-HRP to 1998  $\mu$ L of 1X Blocking Buffer.
  - c). This working dilution can be stored for 3-5 days at 4°C.
5. Detection Buffers C & D are supplied as 1X solutions that are intended to be mixed in a 1:1 ratio *immediately prior use*. Detection reagents may be stored at 4°C for up to 3 months.

## B. Blocking and Incubations

***NOTE: Please prepare all reagents immediately prior to use as described above (Section IV.A) and carefully read tips on Sample Preparation (Section III.A) and Incubations and Washes (Section III.C) before proceeding.***

- 1) Place each membrane printed side up (see Section III.B) into the 8-well tray provided in the kit.
- 2) Block membranes by incubating with 2 mL 1X Blocking Buffer at room temperature (RT) for 30 min.
- 3) Decant Blocking Buffer, and incubate membranes with 1 mL of sample at RT for 1-2 h.

- 4) Aspirate samples from membranes. Wash 3 times, 5 min per wash, with 2 mL Wash Buffer I at RT. Use fresh buffer for each wash.

*OPTIONAL Large Volume Wash: After Step 4, place membranes into clean container(s). Add 20-30 mL of Wash Buffer I per membrane, and wash at RT with gentle shaking or rocking for 30-45 min. Return membranes to the 8-well tray. Then proceed to Step 5.*

- 5) Wash 2 times, 5 min per wash, with 2 mL of 1X Wash Buffer II each at RT. Use fresh wash buffer each time.
- 6) Add 1 mL of 1X Biotin-conjugated Anti-Cytokines to each membrane. Incubate at RT for 1-2 hours.
- 7) Decant or aspirate Anti-Cytokine reagent and repeat washes as described in steps 4 and 5 above.
- 8) Incubate at RT for 2 hours with 1 mL of 1X Streptavidin-HRP.
- 9) Wash membranes as directed in steps 4 and 5.
- 10) Proceed with Detection protocol (below) or store membranes between plastic sheets (provided in kit) as directed in Steps 20 & 21 below.

### C. Chemiluminescence Detection

***NOTE: Do not allow membranes to dry out during detection. Detection of chemiluminescence should be started within 5 minutes after removing Detection Buffers and must be completed within 20 minutes.***

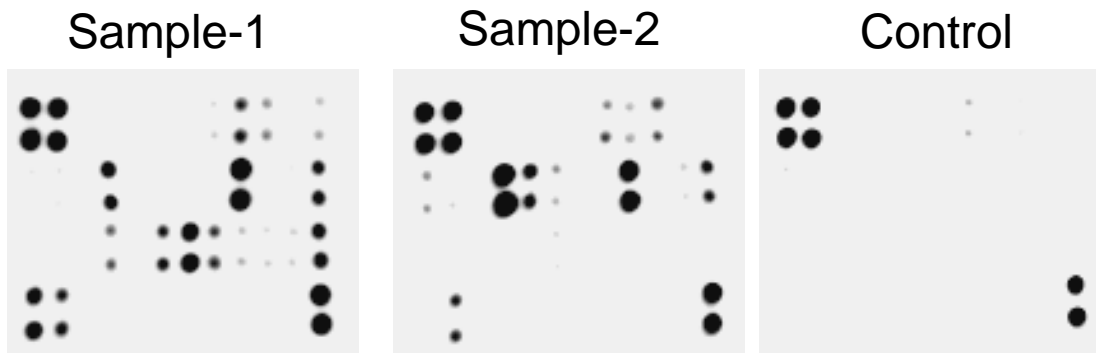
- 11) Place a plastic sheet (provided in the kit) on your benchtop.
- 12) Place one or more array membranes protein side up (see Section III.B) on the plastic sheet. Drain excess liquid by touching one edge to blotting paper or tissue paper.

- 13) Into a single, clean tube add equal volumes of Detection Buffer *C* and Detection Buffer *D* immediately prior to detection. *Mix well*. Add 250  $\mu$ L of each buffer per membrane to be detected, e.g., for 4 membranes, combine 1 mL of each detection buffer.
- 14) Pipette the mixed Detection Buffers onto each membrane. Place another plastic sheet on top, starting at one end and “rolling” the flexible plastic across the surface to the opposite end. During this process, ensure that the detection mixture completely covers each membrane, and gently smooth out any air bubbles. Avoid sliding the plastic sheet along the membranes’ printed surfaces.
- 15) Incubate at RT for 2 minutes.
- 16) Remove top plastic sheet and aspirate excess liquid (see Step 12).
- 17) Gently replace the membranes (protein side up) on the bottom plastic sheet and replace the top plastic sheet (see Step 14). Gently smooth out any air bubbles on the membrane surfaces.
- 18) Detect signals using a chemiluminescence imaging system or expose the array membranes to x-ray film (we recommend Kodak’s X-Omat™ AR film) and develop the film (See tips for obtaining array images in Section III.D).
- 19) For each array, use multiple exposures to obtain an image with low background and strong Positive Control signals that do not bleed into one another. Typical exposure times are 10 seconds to 2 min.
- 20) When you finish your last exposure, remove the top plastic sheet. Gently rinse membranes and plastic sheets with Wash Buffer II. Remove excess wash buffer as described in Step 14, and replace the membranes between the plastic sheets.

- 21) Wrap the sheets in Saran Wrap, and store the membranes at -20°C to -80°C. (Or store membranes for up to 5 days at 4°C in Wash Buffer II. Cover the container to avoid evaporation.)

#### V. Interpretation of Results:

##### Typical results obtained with RayBio® C Series Antibody Arrays



The preceding figure presents typical images obtained with RayBio® Human Cytokine Antibody Array. These membranes were probed with conditioned media from two different cell lines. Membranes were exposed to Kodak X-Omat® film at RT for 1 min.

Note the strong signals of the Positive Control spots, provided by biotin-conjugated IgG printed directly onto the array membrane in the upper-left and lower-right corners. These Positive Control spots are useful for proper orientation of the array image.

The signal intensity for each antigen-specific antibody spot is proportional to the relative concentration of the antigen in that sample. Comparison of signal intensities for individual antigen-specific antibody spots between and among array images can be used to determine relative differences in expression levels of each analyte sample-to-sample or group-to-group.

#### Obtaining Densitometry Data:

Visual comparison of array images may be sufficient to see differences in relative protein expression. However, most researchers will want to perform numerical comparisons of the signal intensities (or more precisely, signal *densities*), using 2-D

densitometry. Gel/Blot documentation systems and other chemiluminescent or phosphorescent detection systems are usually sold as a package with compatible densitometry software.

To obtain densitometry data from an X-ray film, one must first scan the film to obtain a digitized image using an ordinary office scanner with resolution of 300 dpi or greater. Any densitometry software should be sufficient to obtain spot signal densities from your scanned images. One such software program, ImageJ, is available for free from the NIH (for more info, visit <http://rsbweb.nih.gov/ij/>).

We suggest using the following guidelines when extracting densitometry data from our array images:

- For each array membrane, identify a single exposure that the exhibits low background signal intensity and strong Positive Control signals that do not “bleed” into one another. Exposure times do not need to be identical for each array, but Positive Control signals on each image should have similar intensities.
- Measure the density of each spot using a circle that is roughly the size of one of the largest spots. Be sure to use the same circle (area and shape) for measuring the signal densities on every array for which you wish to compare the results.
- For each spot, use the summed signal density across the entire circle (i.e., total signal density per unit area)

Before analysis, subtract the background from raw densitometry data and normalize the signal intensities to the Positive Controls.

#### Background Subtraction:

On each array, several “Negative Control” and/or “Blank” spots will be included. Blank spots are literally blank; nothing has been printed there. Negative Control spots are printed with the same buffer used to dilute antibodies printed on the array. Thus, the signal intensities of the Negative Controls represent the



background plus non-specific binding to the printed spots. We recommend subtracting the mean of 4 or more Negative Control spots for background correction.

### Normalization of Array Data:

The amount of biotin-conjugated IgG protein printed for each Positive Control spot is consistent from array to array. As such the intensity of these Positive Control signals can be used to normalize signal responses for comparison of results across multiple arrays, much like housekeeping genes and proteins are used to normalize results of PCR gels and Western Blots, respectively.

To normalize array data, one 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 first column on the left of 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 density of Positive Control spots on reference array

P(y) = mean signal density of Positive Control spots on Array "y"

X(y) = mean signal density for spot "X" on Array for sample "y"

X(Ny) = normalized signal intensity for spot "X" on Array "y"

After background subtraction and normalization, you can compare signal intensities, analyte-by-analyte, among or between samples or groups to determine relative differences in cytokine expression.

The RayBio<sup>®</sup> Analysis Tool software is available for use with data obtained using RayBio<sup>®</sup> Cytokine Antibody Arrays. Copy and paste your signal intensity data into the "Aligning Data" worksheet, and it will compile and organize your data, as well as automatically subtracting background signals and normalizing to

the Positive Controls. To order the Analysis Tool, please contact us at +1-770-729-2992 or [info@raybiotech.com](mailto:info@raybiotech.com) for more information.

## VI. RayBio® C Series Rat Acute Kidney Injury Antibody Array 1 Maps:

### Detects 7 cytokines in one experiment

	A	B	C	D	E	F	G	H
1	POS	POS	NEG	NEG	Cystatin C	FABP1	KIM-1	MCP-1
2	POS	POS	NEG	NEG	Cystatin C	FABP1	KIM-1	MCP-1
3	NGAL	TIMP-1	VEGF	NEG	NEG	NEG	NEG	POS
4	NGAL	TIMP-1	VEGF	NEG	NEG	NEG	NEG	POS

#### Abbreviations:

POS = Positive Control, NEG = Negative Control, L-FABP = Liver Fatty-Acid Binding Protein, KIM-1 = Kidney Injury Molecule-1, NGAL= Neutrophil Gelatinase-Associated Lipocalin (Lipocalin 2). All others use standard abbreviations.



POS	1,2,9,10,24,32
Cystatin C	5,13
FABP1	6,14
KIM-1	7,15
MCP-1	8,16
NGAL	17,25
TIMP-1	18,26
VEGF	19,27
NEG	3,4,11,12,20,28,21,29,22,30,23,31

## VII. Troubleshooting guide

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
No signal for any spots, including Positive Controls	Global detection failure	Repeat incubation with HRP-Streptavidin and Detection Buffers
Weak or no signals antigen-specific spots	Sample is too dilute	Repeat experiment using higher sample concentration
	Improper dilution of HRP-Streptavidin	Tube may contain precipitants. Repeat detection, mix 1000X HRP-Streptavidin well before diluting reagent
	Waiting too long to detect chemiluminescent signals	Repeat detection, making sure to complete this process within 20 min.
	Other Tips	Incubate with sample O/N at 4°C
		Increase concentration of HRP-Streptavidin
		Increase concentration of Biotin-conjugated Anti-Cytokine
		Extend exposure time (may go overnight)
Uneven signal or background	Bubbles present on membrane during incubations	Be sure to completely remove all bubbles from membrane surface
	Membranes were not evenly covered during washes/incubations or allowed to dry out	Completely cover membranes with solution, use a rocker or shaker during washes and incubations
High background signals	Overexposure	Decrease exposure time
	Sample is too concentrated	Repeat experiment using more dilute sample
	NOTE: To reduce background on completed membrane, wash O/N @ 4°C in Wash Buffer II, then re-incubate with HRP-Streptavidin and repeat detection.	

## VIII. Selected References Featuring RayBio® C-Series Arrays

1. Ray S, Britschgi M, Herbert C, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nature Med.* 2007; 13(11):1359-1362.
2. McAllister SS, Gifford AM, Greiner AL, et al. Systemic Endocrine Instigation of Indolent Tumor Growth Requires Osteopontin. *Cell.* 2008; 133: 994-1005.
3. Acosta JC, O'Loughlen A, Banito A, et al. Chemokine Signaling via the CXCR2 Receptor Reinforces Senescence. *Cell.* 2008; 133: 1006-1018.
4. Brown JM, Boysen MS, Chung S, et al. Conjugated Linoleic Acid Induces Human Adipocyte Delipidation: Autocrine/Paracrine Signaling by Adipokines. *J Biol Chem.* 2004; 279(25): 26735-26747.
5. Celis JE, Moreira JMA, Gromova I, et al. Towards discovery-driven translational research in breast cancer. *FEBS J.* 2004;272: 2-15.
6. Lin PW, Simon PO, Gewirtz AT, et al. Paneth Cell Cryptdins Act in Vitro as Apical Paracrine Regulators of the Innate Inflammatory Response. *J Biol Chem.* 2004; 279(19): 19902-19907.
7. Xu Y, Kulkosky J, Acheampong E, et al. HIV-1-mediated apoptosis of neuronal cells: Proximal molecular mechanisms of HIV-1-induced encephalopathy. *PNAS.* 2004; 101(18):7070-7075.
8. Tang X, Marciano DL, Leeman SE, Amar S. LPS induces the interaction of a transcription factor, LPS-induced TNF-alpha factor, and STAT6(B) with effects on multiple cytokines. *PNAS.* 2005; 102(14): 5132-5137.
9. Wang F-X, Xu Y, Sullivan J, et al. IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. *J Clin Invest.* 2005; 155: 128-137.
10. De Ceuninck F, Marcheteau E, Berger S, et al. Assessment of Some Tools for the Characterization of the Human Osteo-arthritic Cartilage Proteome. *J Biomol Tech.* 2005; 16: 256-265.

11. Boucharaba A, Serre C-M, Guglielmi J, et al. The type 1 lysophosphatidic acid receptor is a target for therapy in bone metastases. *PNAS*. 2006; 103(25):9643-9648.
12. Matsunaga K, Yanagisawa S, Ichikawa T, et al. Airway cytokine expression measured by means of protein array in exhaled breath condensate: Correlation with physiologic properties in asthmatic patients. *J Allergy Clin Immunol*. 2006; 118: 84-90.
13. Vargas DL, Nascimbene C, Chitra Krishnan C, et al. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005; 57: 67-81.
14. Miyoshi N, Oubrahim H, Chock PB, Stadtman ER. Age-dependent cell death and the role of ATP in hydrogen peroxide-induced apoptosis and necrosis. *PNAS*. 2006; 103(6): 1727-1731.
15. Coppinger JA, O'Connor R, Wynne K, et al. Moderation of the platelet releasate response by aspirin. *Blood*. 2007; 109: 4786-4792.
16. Cortez DM, Feldman MD, Mummidi S, et al. IL-17 stimulates MMP-1 expression in primary human cardiac fibroblasts via p38 MAPK- and ERK1/2-dependent C/EBP-beta, NF-kB, and AP-1 activation. *Am J Physiol Heart Circ Physiol*. 2007; 293: H3356-H3365.
17. Walt DR, Blicharz TM, Hayman RB, Rissin DM, et al. Microsensor Arrays for Saliva Diagnostics. *Ann NY Acad Sci*. 2007; 1098:389-400.

Customized RayBio® Cytokine Antibody Arrays. Select your cytokines of interest from the following list, and we will produce the customized array for you. For more information, please visit our website, [www.raybiotech.com](http://www.raybiotech.com).

4-1BB	CNTF	GITR	IL-18 BP $\alpha$	MIP-1 $\delta$	SAA
ACE-2	Cripto	GITR Ligand	IL-18 R $\beta$	MIP-3 $\alpha$	sgp130
Acrp30	CRP	GM-CSF	IL-1ra	MIP-3 $\beta$	Shh N
Activin A	CTACK	GRO ( $\alpha/\beta/\gamma$ )	IL-2	MMP-1	Siglec-5
Adiposin	CXCL16	GRO $\alpha$	IL-2 R $\beta$	MMP-10	Siglec-9
Adipsin	DAN	GH	IL-2 R $\gamma$	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
$\alpha$ -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	MPlF-1	TGF $\alpha$
Angiopoietin-2	Dtk	ICAM-1	IL-31	MSP $\alpha$	TGF $\beta$ 1
Angiostatin	E-Cadherin	ICAM-2	IL-4	NAP-2	TGF $\beta$ 2
ANGPTL4	EDA-A2	ICAM-3	IL-5	NCAM-1	TGF $\beta$ 3
Axl	EGF	IFN $\gamma$	IL-5 R $\alpha$	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- $\beta$ 1	Tie-2
BDNF	Endoglin	IGFBP-3	IL-8	NT-3	TIM-1
$\beta$ 2M	Eotaxin	IGFBP-4	IL-9	NT-4	TIMP-1
$\beta$ 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	TNF $\alpha$
BMP-4	ErbB2	IGF-II	LAP	PAI-I	TNF $\beta$
BMP-5	ErbB3	IL-1 $\alpha$	Leptin	PARC	TNFRSF21
BMP-6	EPO R	IL-1 $\beta$	Leptin R	PDGF R $\alpha$	TNFRSF6
BMP-7	E-Selectin	IL-1 R II	LIF	PDGF R $\beta$	TRAIL R2
$\beta$ -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 R $\alpha$	Lymphotactin	PIGF	TSH
CA IX	FGF-6	IL-10 R $\beta$	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 R $\alpha$ -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-1 $\alpha$	SDF-1	
CK $\beta$ 8-1	GDNF	IL-17R	MIP-1 $\beta$	SDF-1 $\beta$	



## Testing Services:

RayBiotech offers full testing services using any of our Array, ELISA or EIA products, including customized products.

*Just send your samples, and we will send you the results.*

## Custom Services:

1. Customized Antibody and Protein Arrays
2. Customized Phosphorylation Arrays
3. Peptide synthesis
4. Peptide arrays
5. Recombinant protein and antibody production
6. ELISA
7. EIA
8. Assay development

## Technology Transfer Program:

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



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

The RayBio<sup>®</sup> C-Series Cytokine Antibody Array is a patent-pending technology developed by RayBiotech.

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 6 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.

X-Omat<sup>™</sup> is the trademark of The Kodak Company.

ChemiDoc-It<sup>®</sup> is the registered trademark of UVP.

**This product is for research use only.**



*©2011 RayBiotech, Inc.*