RayBio® Biotin Label-based Rat Antibody Array 1

For the Simultaneous Detection of the Relative Expression of 90 Rat Proteins in Cell Culture Supernates, Serum or Plasma

Cat#: AAR-BLG-1-4 (1 glass chip with 4 sub-arrays) and AAR-BLG-1-8 (2 glass chips with 8 sub-arrays)

User Manual (Revised Jun 27, 2011)

Please read manual carefully before starting experiment

As the Protein Array Pioneer Company, Excellence and Innovation Is Our Goal

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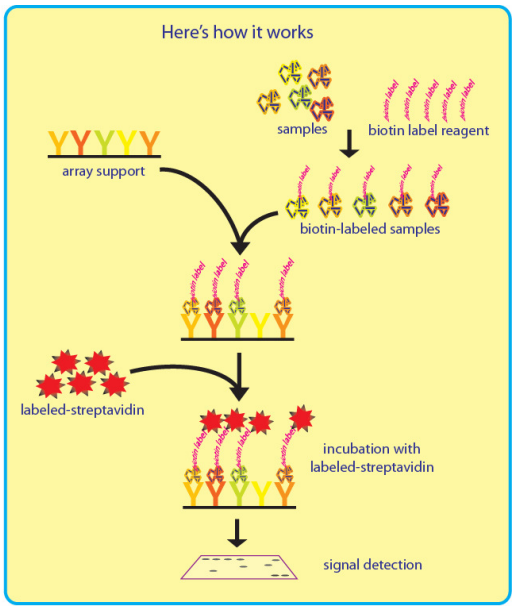
RayBiotech, Inc

TABLE OF CONTENTS

I. Introduction

Recent technological advances by Raybiotech have enabled the largest commercially available antibody array to date. With the L Series 90, researchers can now obtain a broad, panoramic view of cytokine expression. The expression levels of 90 rattarget proteins can be simultaneously detected, including cytokines, chemokines, adipokine, growth factors, angiogenic factors, proteases, soluble receptors, soluble adhesion molecules and other proteins in cell culture supernates, serum and plasma.

The first step in using the RayBio® Biotin Label-based Rat Antibody Array 1 is to biotinylate the primary amine of the proteins in cell culture supernate, serum or plasma samples. The glass chip arrays are then blocked, just like a Western blot, and the biotin-labeled sample is added onto the glass chip, pre-printed with capture antibodies, and incubated to allow for interaction of target proteins. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then applied to the array. Finally, the glass chip is dried, and laser fluorescence scanning is used to visualize the signals.



II. Materials Provided

Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, the Blocking Buffer, Stop Solution, 20X Wash Buffer I and II, Serum Buffer and Streptavidin-conjugated fluorescent dye should be stored at 4 °C to avoid repeated freeze-thaw cycles. The glass chip array should be kept at -20 °C.

- Dialysis tube (Item A, 8 tubes for 4-subarrary chips, and 16 for 8-subarray chips)
- Labeling Reagent (Item B, 2 tube per 4 sub-arrays)
- Stop Solution (Item D, 50 µl)
- Glass chip with chamber assembly for RayBio® Biotin Label-based Rat Antibody Array 1 (Item E, 4 Sub-arrays per glass chip). Kits may contain 1 glass chip for 4 samples or 2 glass chips for 8 samples.
- Blocking Buffer (Item F, 8 ml)
- 20X Wash Buffer I (Item G, 30ml)
- 20X Wash Buffer II (Item H, 30ml)
- Streptavidin-conjugated Fluorescent dye; HiLyte Plus™ 532 (Item I, Cy3 equivalent, 1 tube per 4 sub-arrays)
- Adhesive film (Item J)
- Serum Buffer (Item K, 8 ml)
- D-Tube Floating Rack (Item L)
- 30 ml centrifuge tube (Item M)

III. Additional Materials Required

- Distilled or de-ionized water
- KCl, NaCl, KH_2PO_4 and Na_2HPO_4 to make dialysis buffer

- Small plastic or glass containers
- Orbital shaker or oscillating rocker
- 1 ml tube
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection
- Aluminum foil

IV. Overview and General Considerations

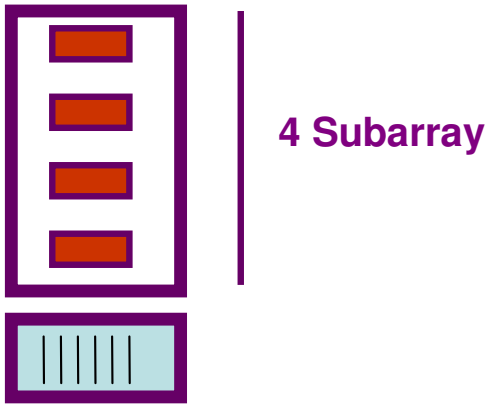
A. Handling glass chips

- The microarray slides are sensitive, so do not touch the array surface with your fingers or pipette tips. Hold the slides by the edges only.
- Handle the slides with powder-free gloves and in a clean environment.
- Do not remove the glass chip from the chamber assembly until step 19, and avoid breaking the glass slide when doing so.
- After each incubation and wash step, remove remaining liquid by gently applying suction with a pipette to corners of each well. Do not touch the printed area of arrays, only the sides.

B. Incubation of Antibody Array

- Cover incubation chamber with adhesive film (included in kit) to prevent evaporation, particularly during incubations or wash steps lasting 2 hours or longer.
- During incubation and wash steps, avoid foaming and be sure to remove all bubbles from the array surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/s).
- Wash steps with Wash Buffer II and all incubation steps may be performed overnight at 4°C.
- Avoid cross-contamination of samples to neighboring wells.
- To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Chip Assembly to decant, and aspirate the remaining liquid.

C. Layout of Array Glass Chip



D. Preparation of Cell Culture Supernates

1). Plate cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes(*).
2) Cultured in complete culture medium for ~24–48 hours (**).
3) Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then the cells are cultured again for ~48 hours (**, †)
4) To collect supernates, centrifuge at 1,000 g for 10 min and store as ≤1 ml aliquots at -80 °C until needed.
5) Measure the total wet weigh of cultured cells in the pellet and culture dish. You may then normalize between arrays by dividing fluorescent signals by total cell mass (ie, express results as amount of protein expressed/mg total cell mass).
6) Determine the total protein concentration immediately prior to biotin labeling (Step 3). We recommended using a BCA total protein assay (eg, Pierce, Catalog # 23227).

*Notes: * The density of cells per dish used is dependent on the cell type. More or less cells may be required.*

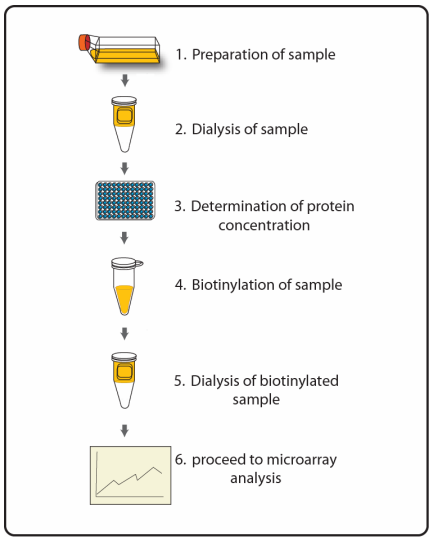
** *Optimal culture time may be different and depends on your cell lines, treatment conditions and other factors.*

† *Bovine serum proteins produce detectable signals on the RayBio® Rat Label-based Antibody Array in uncultured media containing serum concentrations as low as 0.2%. When testing serum-containing media with label-based arrays, it is necessary to also test an uncultured media blank for comparison with sample results.*

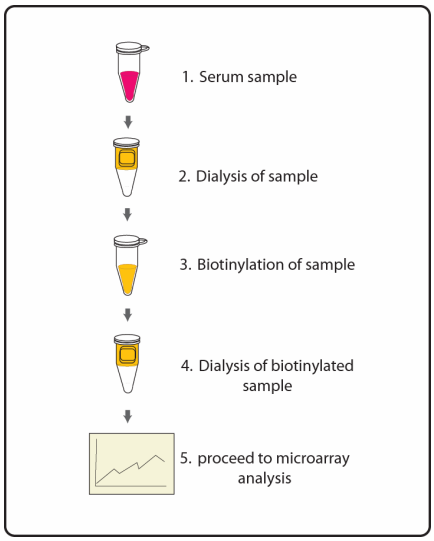
V. Protocol

Assay Diagram

1. Cell Culture Supernates



2. Serum or plasma



Dialysis of Sample

Note: Samples must be dialyzed prior to the biotin-labeling.

1. To prepare dialysis buffer (1X PBS, pH=8.0), dissolve 0.6 g KCl, 24 g NaCl, 0.6 g KH_2PO_4 and 3.45 g Na_2HPO_4 in 2500 ml de-ionized or distilled water. Adjust to pH=8.0 with 1M NaOH and adjust final volume to 3000 ml with de-ionized or distilled water

2. Add each sample into a separate Dialyzer Tube (Item A). Load 200 µl cell culture supernate or 20 µl serum/plasma + 80 µl 1X PBS, pH=8, (5-fold dilution). Carefully place Dialyzer Tubes into D-Tube Floating Rack.

3. Place D-Tube Floating Rack into ≥500 ml dialysis buffer in a large beaker. Place beaker on a stir plate and dialyze at least 3 hours at 4 °C, stirring buffer gently. Then exchange the 1X PBS buffer and repeat dialysis for at least 3 h at 4 °C. Transfer dialyzed sample to clean eppendorf tube. Spin biotin-labeled samples for 5 min at 10,000 rpm (4 °C) to remove particulates and precipitants.

Note: The sample volume may change during dialysis.

Biotin Labeling of Samples

Note: Amines (e.g., Tris, glycine) and azides quench the biotinylation reaction. Avoid contaminating samples with these chemicals prior to biotinylation.

3. Immediately before use, prepare 1X Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl 1X PBS into the tube, pipette up and down or vortex slightly to dissolve the lyophilized reagent. Mix well.

4. Add 1X Labeling Reagent to dialyzed samples.

   a) For labeling cell culture supernates**:** transfer 180 µl dialyzed sample into a new tube. Add *36 µl of 1X Labeling Reagent Soluition per 1 mg total protein in dialyzed cell culture supernate*. Mix well.
   b) For labeling serum or plasma: Add 22 µl of 1X Labeling Reagent Solution into a new tube containing 35 µl dialyzed serum or plasma sample and 155 µl Serum Buffer (Item K). Mix well.

Note: To normalize serum/plasma concentrations during biotinylation, measure sample volume before and after dialysis. Then adjust the volumes of dialyzed serum and Serum Buffer to compensate. For example, if serum/plasma sample volume increased from 100 µl to 200 µl, add 70 µl dialyzed serum to 120 µl Serum Buffer.

5. Incubate the reaction solution at room temperature with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 min.

6. Add 3 µl Stop Solution (Item D) into each reaction tube and immediately dialyze as directed in Step B.

7. Transfer dialyzed sample to clean eppendorf tube.

Note: Biotinylated samples may be stored at -20 °C or -80 °C until you are ready to proceed with the assay.

Dry the Glass Chip

8. Remove the package containing the glass chip assembly from the freezer. Place unopened package on the benchtop and allow the glass chip assembly to equilibrate to room temperature (RT), approx. 15 min.

9. Open package, take glass chip assembly out from box (do not remove the glass chip from the chamber assembly) and dry it for 1-2 hours at RT in clean environment.

Note: Protect the chip from dust or others contaminants.

Blocking and Incubation of Antibody Array

Note: Glass chip should be completely dry before proceeding.

10. Block sub-arrays by adding 400 µl of Blocking Buffer (Item F) into each well of glass chip assembly (Item E) and incubate at RT for 30 min with gentle rocking or shaking. Make sure there are no bubbles on the array surfaces.

11. Immediately prior to sample incubation, spin biotin-labeled samples for 5 min at 10,000 rpm to remove particulates and precipitants. Dilute samples with Blocking Buffer.*

**Note: Recommended dilution of the biotin-labeled samples with Blocking Buffer prior to incubation is 2-10 fold for cell culture supernates or 20-fold for serum/plasma.*

12. Completely remove Blocking Buffer from each well. Add 400 µl of diluted samples into appropriate wells. Remove any bubbles on the array surfaces. Incubate with gentle rocking or shaking for 2 hours at RT or overnight at 4 °C.

Note: Optimal dilution will depend on the abundance of target proteins. If the background or antigen-specific antibody signals are too strong, the sample can be diluted further in subsequent experiments. If the signal is too weak, more concentrated samples can be used.

Note: Avoid the flow of sample into neighboring wells.

13. Dilute 20X Wash Buffer I (Item G) 20-fold with de-ionized or distilled water. Decant the samples from each well, and

wash 3 times with 800 µl of 1X Wash Buffer I at RT with gentle rocking or shaking for 5 min per wash.

14. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the glass chip assembly into the box with sufficient 1X Wash Buffer I to cover the entire assembly, and remove all bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 10 min per wash.

15. Dilute 20X Wash Buffer II (Item H) 20-fold with de-ionized or distilled water. Decant the Wash Buffer I from each well, place the glass chip assembly into the box with sufficient 1X Wash Buffer II to cover the entire assembly, and remove all bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 5 min per wash.

16. Prepare Streptavidin-conjugated Fluorescent Dye:
    a) Briefly spin down the Streptavidin-conjugated Fluorescent Dye (Item I) immediately before use.
    b) Add 1000 µl of Blocking Buffer into the tube to prepare a concentrated Streptavidin-Fluor stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).
    c) Add 200 µl of Streptavidin-Fluor concentrate into a tube with 800 µl of Blocking Buffer. Mix gently to prepare 1X working dilution.

17. Carefully remove glass slide assembly from container. Remove all of Wash Buffer II from the wells. Add 400 µl of 1X Streptavidin-conjugated Fluorescent Dye to each sub-array. Cover the incubation chamber with adhesive film.

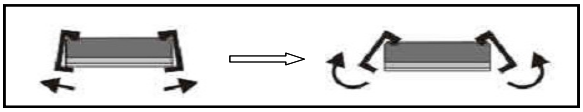
Note: Avoid exposure to light in steps 18–24.

18. To avoid exposure to light, cover the glass chip assembly with aluminum foil or incubate in dark room. Incubate at room temperature for 2 hours with gentle rocking or shaking.

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

19. Decant the solution and disassemble the glass chip from the incubation frame and chamber. Disassemble the device by pushing clips outward from the side. Carefully remove the glass chip from the gasket.

Note: Be careful not to touch the printed surface of the glass chip, which is on the same side as the barcode.



20. Gently place the glass chip into a 30 ml centrifuge tube provided in the kit and add enough 1X Wash Buffer I to cover the entire glass chip. Wash with gentle rocking or shaking for 10 min. Remove the wash buffer. Repeat wash 2 times for a total of 3 washes.

21. Repeat step 19, this time with 1X Wash Buffer II. Repeat one time for a total of two washes for 5 min per wash.

22. Finally wash the glass chip with 30 ml of de-ionized or distilled water for 5 min. Remove glass chip and decant water from centrifuge tube.

23. Remove excess liquid from centrifuge tube, and place glass chip into the tube. Centrifuge at 1,000 rpm for 3 minutes to

remove water droplets. Make sure the slides are completely dry before scanning or storage.

Note: Alternatively, you may gently dry the glass chip using a low-velocity Nitrogen gas stream. You may also air dry the glass chip dry in a clean environment (Be sure protect from light).

Fluorescence Detection

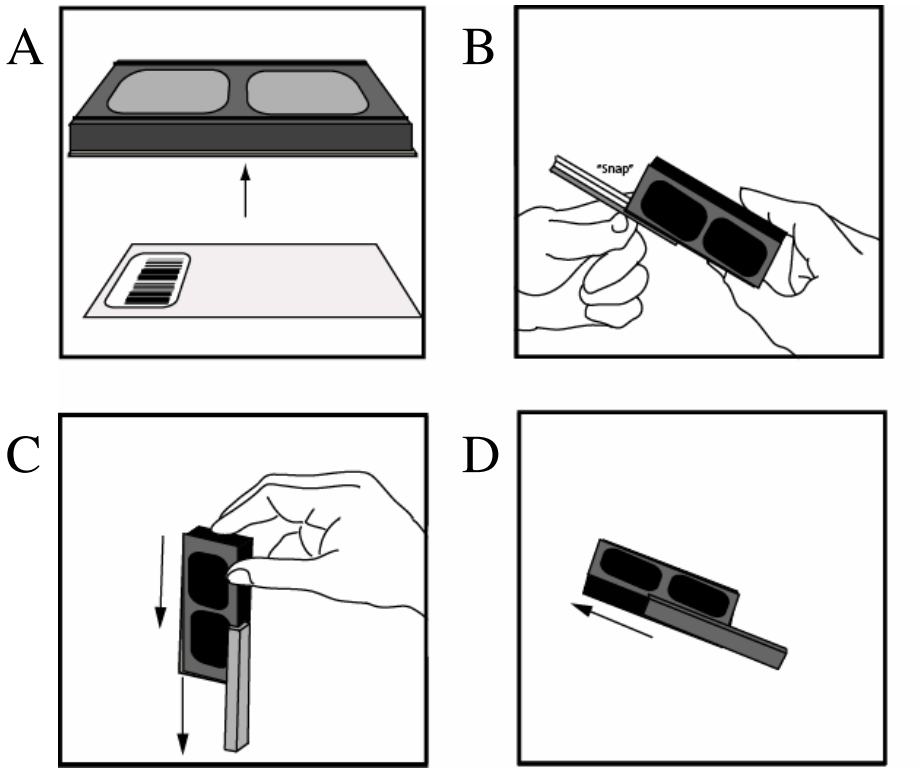
24. You may proceed immediately to laser fluorescence scanning, or you may store the slide at -20 °C in the centrifuge tube provided or at RT and to scan at a later time.

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 temperatures above RT and store them in the dark. Do not expose glass chip to strong light, such as sunlight or UV lamp.

Note: If you need to repeat any of the incubation after finishing the experiment, you must first re-assemble the glass chip into the incubation chamber by following step as shown in the figures below. You may first want to practice assembling the device with a blank glass slide.

1. *Apply slide to incubation chamber barcode facing upward as in image A (below).*
2. *Gently snap one edge of a snap-on side as shown in image B.*

3. *Gently press other of side against lab bench and push in lengthwise direction, as shown in image C.*
4. *Repeat with the other side (image D).*



VI. Interpretation of Results:

A. Explanation of Controls Spots

1) **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

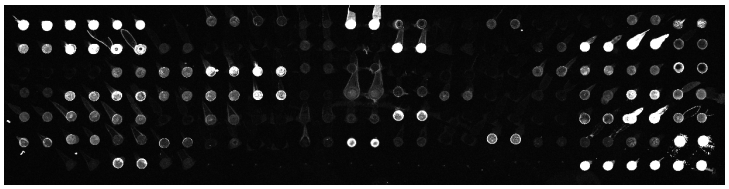
"housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

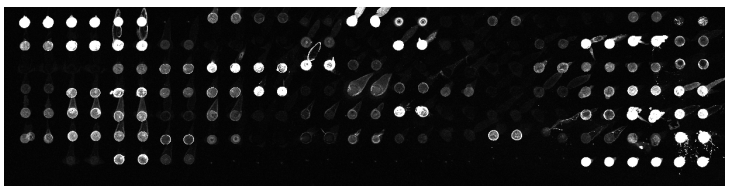
2) **Negative Control (NEG) spots** are a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of Biotin-conjugated anti-Cytokines and/or Steptavidin-Fluor. Negative control signal intensities are usually very close to background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

3) **Blank spots** are literally "blank," meaning that nothing is printed there.

B. Typical results obtained with RayBio® Biotin Label-based Rat Antibody Array 1

The following figure shows the RayBio® **Biotin-label-based Rat Antibody Array 1** probed with serum sample. The images were captured using a Axon GenePix laser scanner.





Note the strong Positive Control signals in the upper left and lower right corners of each array, which can be used to identify the orientation and help normalize the results between arrays. If scanned using optimal 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.

Antigen affinities vary significantly between antibodies. The fluorescence intensity detected on the array with each antibody depends on its affinity; therefore, signal intensity comparison can be performed only for individual antibody/antigen signals between samples and not between different antibody/antigen signals within an array.

Also, in the absence of an external standard curve for each protein detected, there is no means of assessing absolute or relative concentrations of different proteins 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® Arrays instead.

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 is available for use with data obtained using RayBio® Biotin Label-based Antibody Arrays. 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 between and array images can be used to determine relative differences in expression levels of each protein 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 is considered a measurable, significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy ≈ 95%).

RayBio® Biotin Label-based Rat Antibody Array 1 List

	Target protein		Target protein		Target protein		Target protein
1	Positive 1	28	Fas Ligand/TNFSF6	55	IP-10	82	TGF-beta3
2	Positive 2	29	FGF-BP	56	Leptin (OB)	83	Thrombospondin
3	Positive 3	30	Follostatin-like -1(FSL1)	57	LIX	84	TIE-2
4	Neg	31	Fractalkine	58	L-Selectin/CD62L	85	TIMP-1
5	Activin A	32	GFR alpha-1	59	MCP-1	86	TIMP-2
6	ACTH	33	GFR alpha-2	60	MDC	87	TIMP-3
7	ADFP	34	GM-CSF	61	MIF	88	TLR4
8	Adiponectin/Acrp30	35	Growth Hormone	62	MIP-1 alpha	89	TNF-alpha
9	AMPK alpha 1	36	Growth Hormone R	63	MIP-2	90	TRAIL
10	B7-1/CD80	37	Hepassocin	64	MIP-3 alpha	91	TROY
11	BDNF	38	ICAM-1/CD54	65	MMP-2	92	Ubiquitin
12	beta-Catenin	39	ICK	66	MMP-8	93	VEGF
13	basic-FGF	40	IDE (Insulin Degrading Enzyme)	67	MMP-13	94	VEGF-C
14	beta-NGF	41	IFN-gamma	68	MuSK	95	Neg
15	CCR4	42	IL-1 alpha	69	Neuropilin-2	96	Neg
16	CD106	43	IL-1 beta	70	NGFR	97	Neg
17	CINC-2 alpha/beta	44	IL-1 R6/IL-1 R rp2	71	Orexin A	98	Neg
18	CINC-3	45	IL-2	72	Osteopontin/SPP1	99	Neg
19	CNTF	46	IL-3	73	PDGF-AA	100	Neg
20	CNTF R alpha	47	IL-4	74	Prolactin R	101	Neg
21	CSK	48	IL-5	75	RAGE	102	Neg
22	CXCR4	49	IL-6	76	RALT/MIG-6	103	Positive 3
23	EGFR	50	IL-10	77	RELM beta	104	Positive 2
24	EG-VEGF/PK1	51	IL-12/IL-23 p40	78	Resistin	105	Positive 1
25	E-Selectin	52	IL-13	79	TAL1A		
26	FADD	53	Integrin alpha M beta 2	80	TGF-beta1		
27	Fas/TNFRSF6	54	Insulin	81	TGF-beta2		

RayBio® Biotin Label-based RatAntibody Array 1 Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Positive 1	Positive 1	Positive 2	Positive 2	Positive 3	Positive 3	Neg	Neg	Activin A	Activin A	ACTH	ACTH	ADFP	ADFP	Adiponectin/Acrp30
2	CD106	CD106	CINC-2 alpha/beta	CINC-2 alpha/beta	CINC-3	CINC-3	CNTF	CNTF	CNTF R alpha	CNTF R alpha	CSK	CSK	CXCR4	CXCR4	EGFR
3	Fractalkine	Fractalkine	GFR alpha-1	GFR alpha-1	GFR alpha-2	GFR alpha-2	GM-CSF	GM-CSF	Growth Hormone	Growth Hormone	Growth Hormone R	Growth Hormone R	Hepassocin	Hepassocin	ICAM-1/CD54
4	IL-3	IL-3	IL-4	IL-4	IL-5	IL-5	IL-6	IL-6	IL-10	IL-10	IL-12/IL-23 p40	IL-12/IL-23 p40	IL-13	IL-13	Integrin alpha M beta 2
5	MIF	MIF	MIP-1 alpha	MIP-1 alpha	MIP-2	MIP-2	MIP-3 alpha	MIP-3 alpha	MMP-2	MMP-2	MMP-8	MMP-8	MMP-13	MMP-13	MuSK
6	RALT/MIG-6	RALT/MIG-6	RELM beta	RELM beta	Resistin	Resistin	TALIA	TALIA	TGF-beta1	TGF-beta1	TGF-beta2	TGF-beta2	TGF-beta3	TGF-beta3	Thrombospondin
7	TROY	TROY	Ubiquitin	Ubiquitin	VEGF	VEGF	VEGF-C	VEGF-C	Neg	Neg	Neg	Neg	Neg	Neg	Neg

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Adiponectin/Acrp30	AMPK alpha 1	AMPK alpha 1	B7-1/CD80	B7-1/CD80	BDNF	BDNF	beta-Catenin	beta-Catenin	basic-FGF	basic-FGF	beta-NGF	beta-NGF	CCR4	CCR4
EGFR	EG-VEGF/PK1	EG-VEGF/PK1	E-Selectin	E-Selectin	FADD	FADD	Fas/TNFRSF6	Fas/TNFRSF6	Fas Ligand/TNFSF6	Fas Ligand/TNFSF6	FGF-BP	FGF-BP	Follostatin-like -1(FSL1)	Follostatin-like -1(FSL1)
ICAM-1/CD54	ICK	ICK	IDE (Insulin Degrading Enzyme)	IDE (Insulin Degrading Enzyme)	IFN-gamma	IFN-gamma	IL-1 alpha	IL-1 alpha	IL-1 beta	IL-1 beta	IL-1 R6/IL-1 R rp2	IL-1 R6/IL-1 R rp2	IL-2	IL-2
Integrin alpha M beta 2	Insulin	Insulin	IP-10	IP-10	Leptin (OB)	Leptin (OB)	LIX	LIX	L-Selectin/CD62L	L-Selectin/CD62L	MCP-1	MCP-1	MDC	MDC
MuSK	Neuropilin-2	Neuropilin-2	NGFR	NGFR	Orexin A	Orexin A	Osteopontin/SPP1	Osteopontin/SPP1	PDGF-AA	PDGF-AA	Prolactin R	Prolactin R	RAGE	RAGE
Thrombospondin	TIE-2	TIE-2	TIMP-1	TIMP-1	TIMP-2	TIMP-2	TIMP-3	TIMP-3	TLR4	TLR4	TNF-alpha	TNF-alpha	TRAIL	TRAIL
Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Positive 3	Positive 3	Positive 2	Positive 2	Positive 1	Positive 1

VII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak signal	Inadequate detection	Check laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettors 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	Sample is too concentrated	Use more diluted sample
	Excess of streptavidin	Make sure to use the correct amount of streptavidin
	Inadequate detection	Check laser power and PMT parameters
	Inadequate wash	Increase the volume of wash buffer and incubation time
Uneven signal	Bubbles formed during incubation	Avoid bubble formation during incubation
	Arrays are not completely covered by reagent	Completely cover arrays with solution

VIII. Reference List

1. Christina Scheel et all. Paracrine and Autocrine Signals Induce and Maintain Mesenchymal and Stem Cell States in the Breast. Cell. 2011;145, 926–940

2. Lin Y, Huang R, Chen L, et al. Profiling of cytokine expression by biotin-labeled-based protein arrays. Proteomics. 2003, 3: 1750–1757.

3. Huang R, Jiang W, Yang J, et al. A Biotin Label-based Antibody Array for High-content Profiling of Protein Expression. Cancer Genomics Proteomics. 2010; 7(3):129–141.

4. Liu T, Xue R, Dong L, et al. Rapid determination of serological cytokine biomarkers for hepatitis B–virus-related hepatocellulare carcinoma using antibody arrays. Acta Biochim Biophys Sin. 2011; 43(1):45–51.

5. Cui J, Chen Y, Chou W-C, et al. An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer. Nucl Acids Res. 2011; 39(4):1197–1207.

6. Jun Zhong et all. Temporal Profiling of the Secretome during Adipogenesis in Humans. Journal of Proteome Research. 2010, 9, 5228–5238

7. Chowdury UR, Madden BJ, Charlesworth MC, Fautsch MP. Proteomic Analysis of Human Aqueous Humor. Invest Ophthalmol Visual Sci. 2010; 51(10):4921–4931.

8. Wei Y, Cui C, Lainscak M, et al. Type-specific dysregulation of

matrix metalloproteinases and their tissue inhibitors in end-stage heart failure patients: relationshp between MMP-10 and LV remodeling. J Cell Mol Med. 2011; 15(4):773–782.

9. Kuranda K, Berthon C, Lepêtre F, et al. Expression of CD34 in hematopoietic cancer cell lines reflects tightly regulated stem/progenitor-like state. J Cell Biochem. 2011; 112(5):1277–1285.

10. Toh HC, Wang W-W, Chia WK, et al. Clinical Benefit of Allogenic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients. Clin Chem Res. 2009; 15:7726–7736.

11. Zhen Hou. Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosi Biomarkers. 2009;14(8): 604-618.

12. Yao Liang Tang,et al. Hypoxic Preconditioning Enhances the Benefit of Cardiac Progenitor Cell Therapy for Treatment of Myocardial Infarction by Inducing CXCR4. Circ Res. 2009;109:197723

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