RayBio® Biotin Label-based Human Obesity Antibody Array 1

For the Simultaneous Detection of the Relative Expression of 108 Human Proteins in Cell Culture Supernates, Serum or Plasma

Cat#: AAH-BLG-ADI-1-2 and AAH-BLG-ADI-1-4

User Manual (Revised Aug 25, 2011)

Please read manual carefully before starting experiment

RayBiotech, Inc.
the protein array pioneer company

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

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RayBiotech, Inc

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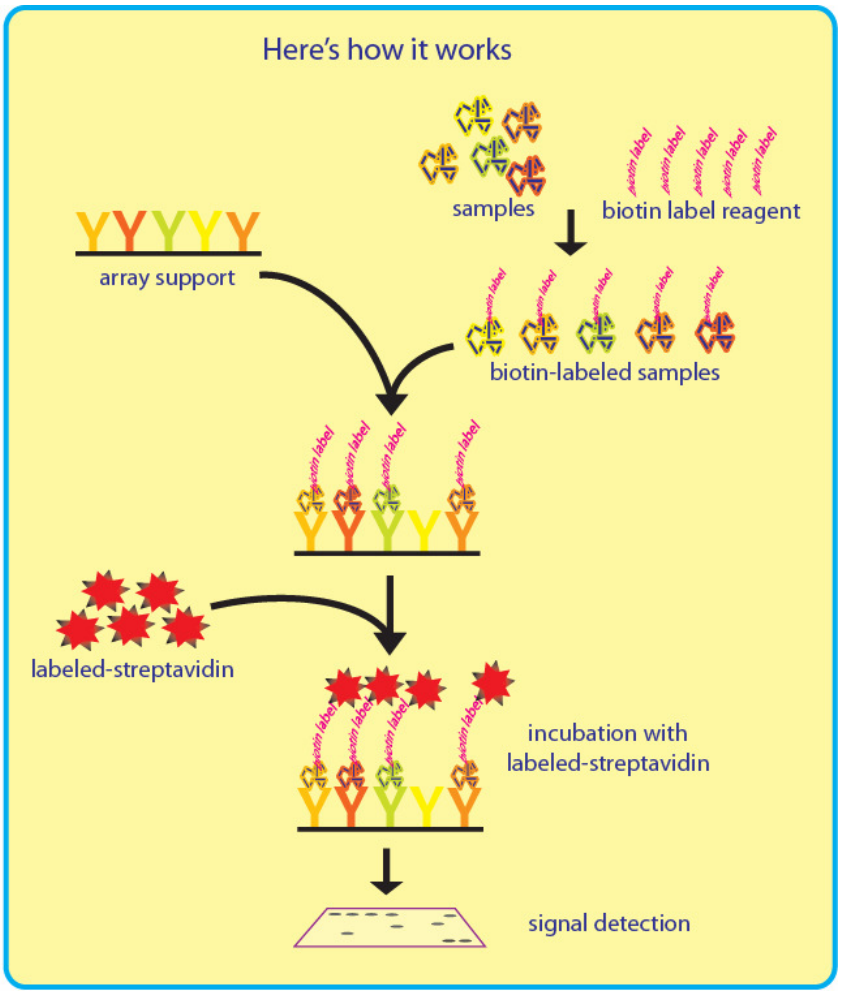
I. Introduction

The area of obesity research is getting hotter ever over the past years. One of the key driving force is that adipose tissue is found no longer to be an inert energy storage organ, but is emerging as an active participant in regulating physiological and pathologic processes. Many soluble factors have been identified from the adipose tissue and are so called as adipocytokines or adipokines. Some of the adipokines are mainly produced by the adipose tissue like leptin and resistin, while others are also synthesized in other tissues like TNF-alpha, IL-6, MCP-1, and IL-1. Because all of these factors can act in an autocrine, paracrine or endocrine manner in the organisms, adipokines are thought to serve as mediators linking obesity, inflammation, immunity and other obesity related diseases.

Recent technological advances by Raybiotech have enabled the largest commercially available antibody array to date. With the L Series Human Obesity Antibody Array 1, researchers can now obtain a broad, panoramic view of adipokine expression. The expression levels of 182 human target proteins can be simultaneously detected in cell culture supernates and serum. Furthermore, an internal control is used to monitor the whole process including biotin-labeling, so this massive array will accurately reflect the available adipokines in your sample.

The first step in using the RayBio® Biotin label-based human obesity antibody array 1 is to biotinylate the primary amine of the proteins in cell culture supernates and serum. The biotin-labeled sample is then added onto glass chip and incubated at room temperature. Fluorescent dye-Conjugated Streptavidin (cy3 equivalent) is used to visualize the signals.



II. Materials Provided

Upon receipt, the kit should be stored at -20°C until needed. Please use within 6 months from the date of shipment. After initial use, remaining reagents should be stored at 4°C to avoid repeated freeze-thaw cycles. Unused glass chips should be kept at -20 °C.

- Dialysis tube (Item A, 4 tubes for AAH-BLG-ADI-1-2 or 8 tubes for AAH-BLG-ADI-1-4)
- Labeling Reagent (Item B, 1 tube for AAH-BLG-ADI-1-2 or 2 tubes for AAH-BLG-ADI-1-4)

- Stop Solution (Item D, 50 µl)
- RayBio® Biotin Label-based Human Obesity Antibody Array 1 Glass Chip in Chamber Assembly (Item E, 2 sub-arrays or 4 sub-arrays in one glass chip). 2 sub-arrays for AAH-BLG-ADI-1-2 or 4 sub-arrays for AAH-BLG-ADI-1-4).
- Blocking Buffer (Item F, 8 ml)
- 20X Wash Buffer I (Item G, 30 ml)
- 20X Wash Buffer II (Item H, 30 ml)
- HiLyte Plus™ 532 Streptavidin-conjugated Fluorescent dye (Item I, Cy3 equivalent, 1 tube per glass chip)
- 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
- Small plastic or glass containers
- Orbital shaker or oscillating rocker
- Beaker, stir plate and stir bar
- 1 ml tube
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection (list of compatible scanners available at http://www.raybiotech.com/resources.asp)
- Aluminum foil

IV. Overview and General Considerations

A. Handling glass chips

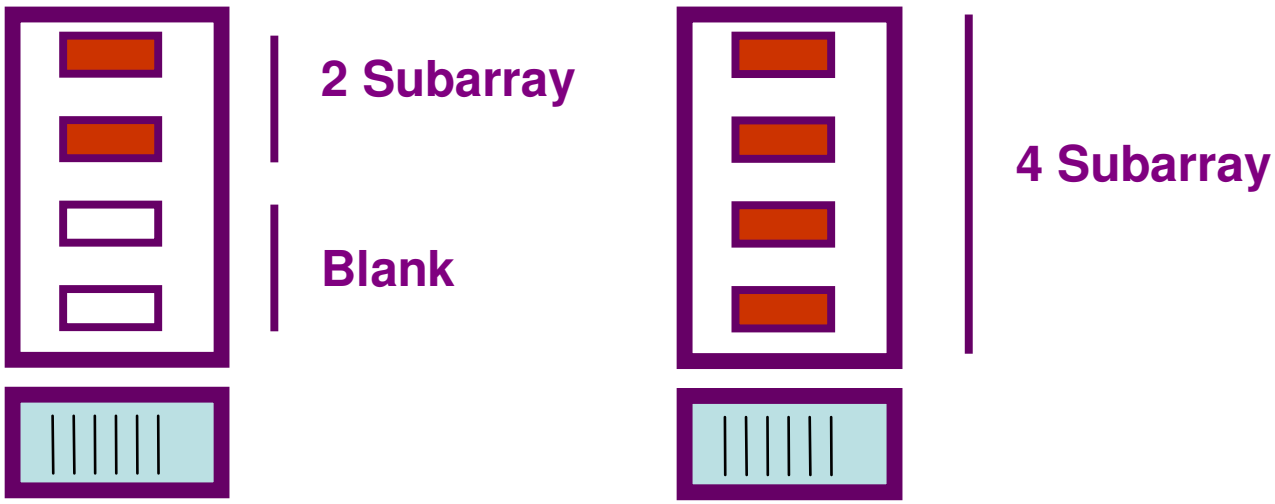
- The microarray slides are delicate. Please do not touch the array surface with pipette tips, forceps or your fingers. Hold the slides by the edges only.
- Handle the slides with powder-free gloves and in a clean environment.
- Do not remove the glass chip from the chamber assembly until step 19, and take great care not to break the glass chip when doing so.
- Remove the final buffer by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides.

B. Incubation of Antibody Array

- Cover incubation chamber with adhesive film (Item J) to prevent evaporation during incubation or wash steps, particularly those lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and be sure to remove all bubbles from the sub-array surface.
- 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.

- 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.
- 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.

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 incubate cells 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 weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can nomalize between array by determining cell lylate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227).

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

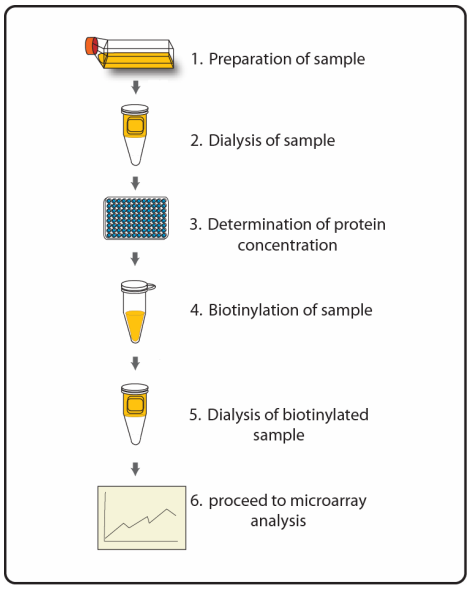
** *Optimal culture time may be different and depends on your cell lines, treatment conditions and other factors.*

† *Bovine serum proteins produce detectable signals on the RayBio® Human Obesity Label-based Antibody Array in media containing serum concentrations as low as 0.2%. When testing serum-containing media, we strongly recommend testing an uncultured media blank for comparison with sample results.*

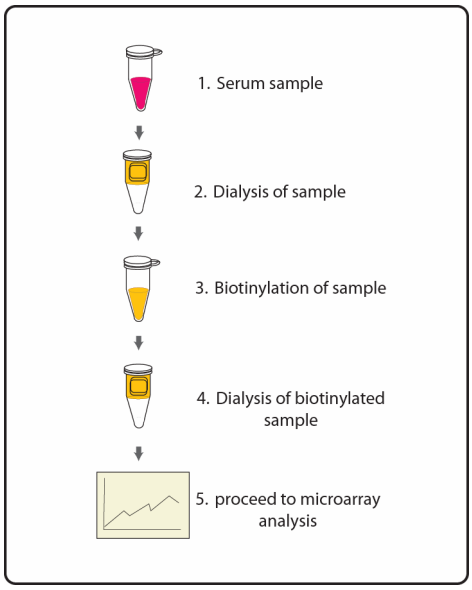
V. Protocol

Assay Diagram

1. Cell Culture Supernates



2. Serum or plasma



Dialysis of Sample

Note: Samples must be dialyzed prior to biotin-labeling (Steps 5–7).

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

3. Place D-Tube Floating Rack into ≥500 ml dialysis buffer in a large beaker. Place beaker on a stir plate and dialyze, for 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 a clean eppendorf tube. Spin dialyzed samples for 5 min at 10,000 rpm to remove any particulates or precipitants, and then transfer the supernates to a clean tube.

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

Biotin-labeling Sample

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

4. 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.

5. 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 Solution per 1 mg total protein in dialyzed cell culture supernate*.* Mix well.

** Note: Determine the total protein concentration immediately prior to biotin labeling (Step 5). We recommended using a BCA total protein assay (eg, Pierce, Catalog # 23227).*

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).

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 and 120 µl Serum Buffer.

6. 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.

7. Add 3 µl Stop Solution (Item D) into each reaction tube and immediately dialyze as directed in Steps 2–3.

Note: Biotinylated samples can 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 (Item E) from the freezer. Place unopened package on the benchtop for approx. 15 min, and allow the Glass Chip Assembly to equilibrate to room temperature (RT).

9. Open package, and take the Glass Chip Assembly out of the

sleeve (Do not remove the Glass Chip from the chamber assembly). Place glass chip assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

Note: Protect the chip from dust or others contaminants.

Blocking and Incubation of Antibody Array

Note: Glass chip should be completely dry before adding Blocking Buffer to wells.

10. Block sub-arrays by adding 400 µl of Blocking Buffer (Item F) into each well of Glass Chip Assembly and incubating at RT for 30 min. Remove any bubbles on the array surfaces.

11. Immediately prior to sample incubation, spin biotin-labeled samples for 5 min at 10,000 rpm to remove any particulates or 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 array surfaces. Incubate arrays with gentle rocking or shaking for 2 hours at RT or overnight at 4°C.

Note: Optimal sample dilution factor 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 Concentrate (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 completely 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 Concentrate (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 completely 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 tube containing 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 <u>not</u> 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 Chip Assembly from containter. 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.

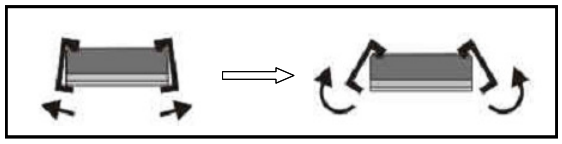
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Chip Assembly with aluminum foil or incubate in dark room.

18. Incubate with Streptavidin-Fluor at RT 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, as shown below. 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 30 ml Centrifuge Tube (Item M). 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 2 times for a total of 3 washes.

21. Repeat step 21, 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 finished glass chip is completely dry before scanning or storage.

Note: Alternatively, you may gently dry the glass chip using a low-velocity Nitrogen gas stream or ambiently in a laminar flow hood or similar clean environment (Be sure to protect from light).

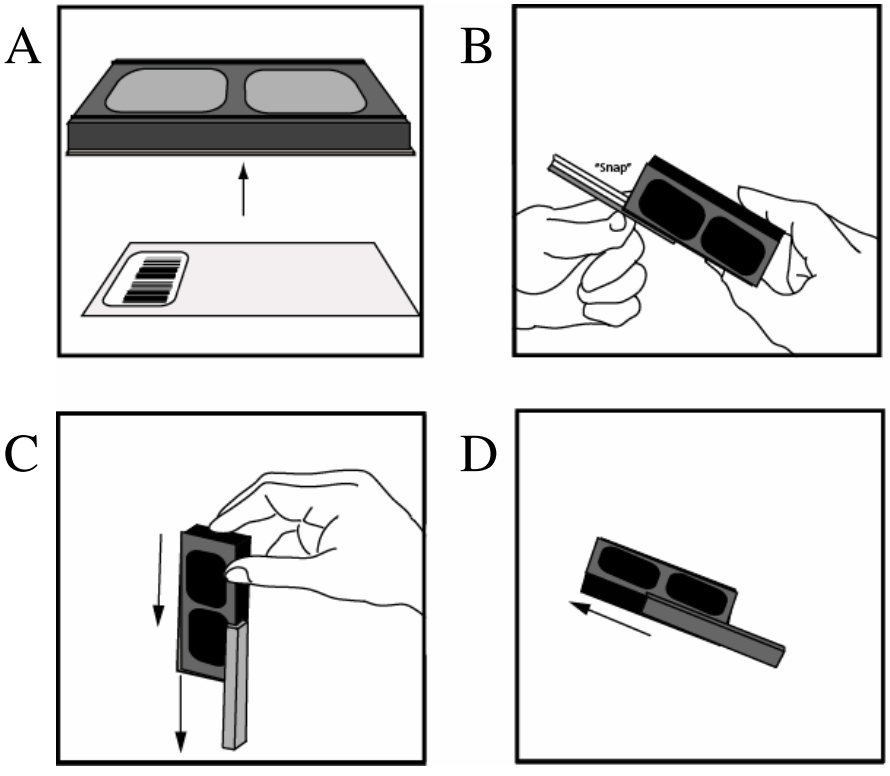
Fluorescence Detection

24. You may proceed immediately to 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. To avoid breaking the printed glass chip, 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 (image C).*
4. *Repeat with the other side (image D).*



VI. Interpretation of Results:

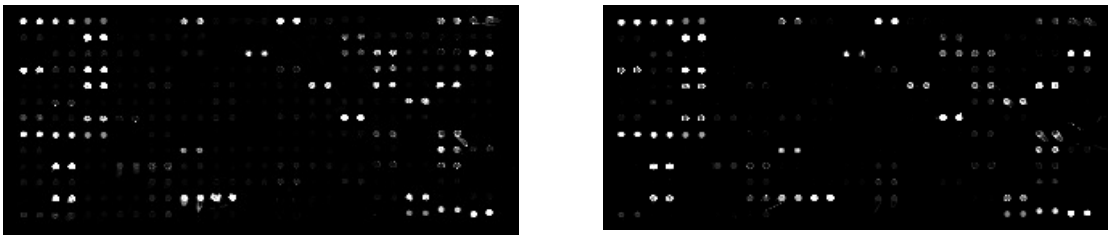
A. Explanation of Controls Spots

1) **Positive Control spots (POS1, POS2, POS3)** are standardized 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** contain 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 the Steptavidin-conjugated Fluor. Negative control signal intensities are usually very close to background signals in each sub-array.

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

The following figure shows the RayBio® **Biotin-label-based Human Obesity Antibody Array 1** probed with serum sample. The images were captured using a Axon GenePix laser scanner. The strong signals in row 8 and the the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays.



If scanned using optimal settings, 3 distinct 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.

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:

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

Most laser fluorescence scanner software have an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEDIAN background signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., 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 is arbitrary. For example, in our Analysis Tool Software (described below), 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® Biotin Label-based Antibody 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 between and among 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 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%).

RayBio® Biotin Label-based Human Adipokine Antibody Array 1 Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	POS-1	POS-1	POS-2	POS-2	POS-3	POS-3	NEG	NEG	ACE / CD43	ACE / CD43	ACE-2	ACE-2	ACTH	ACTH	ADFP
2	Angiotensinogen / Angiotensin II	Angiotensinogen / Angiotensin II	Ang-like Factor	Ang-like Factor	ANGPTL1	ANGPTL1	ANGPTL2	ANGPTL2	ANGPTL3	ANGPTL3	ANGPTL4	ANGPTL4	APJ	APJ	ApoB
3	BMP-4	BMP-4	BMP-5	BMP-5	BMP-6	BMP-6	BMP-7	BMP-7	BMP-8	BMP-8	BMP-15	BMP-15	BMPR-IA / ALK-3	BMPR-IA / ALK-3	BMPR-IB / ALK-6
4	CNTF	CNTF	C-peptide	C-peptide	CRP	CRP	Cystatin C	Cystatin C	Dtk	Dtk	EGF	EGF	EGF-R	EGF-R	ENA-78
5	FGF-10	FGF-10	FGF-6	FGF-6	FSH	FSH	Galectin -1	Galectin -1	GH (Growth Hormone)	GH (Growth Hormone)	Ghrelin	Ghrelin	GITR	GITR	GITRL
6	Glutathione peroxidase 3	Glutathione peroxidase 3	GROa	GROa	HCC4	HCC4	HGF	HGF	HSD-1	HSD-1	ICAM1	ICAM1	IFNg	IFNg	IGF-1
7	IL-1a	IL-1a	IL-1b	IL-1b	IL-1ra	IL-1ra	IL-6	IL-6	IL-6 sR	IL-6 sR	IL-8	IL-8	IL-10	IL-10	IL-11
8	POS-1	POS-1	POS-2	POS-2	POS-3	POS-3	NEG	NEG	Leptin R	Leptin R	LH (Luteinizing Hormone)	LH (Luteinizing Hormone)	LIF	LIF	LOX
9	MIP-3b	MIP-3b	MMP-2	MMP-2	MMP-9	MMP-9	MMP-11	MMP-11	MMP-19	MMP-19	MSHa	MSHa	MSPa	MSPa	Myostatin
10	Orexin B	Orexin B	OSM	OSM	Osteocalcin	Osteocalcin	Osteonectin	Osteonectin	Osteoprotegerin	Osteoprotegerin	PARC	PARC	PDGF-BB	PDGF-BB	PDGF-AA
11	Prohibitin	Prohibitin	Prolactin	Prolactin	PYY	PYY	RANTES	RANTES	RBP4	RBP4	RELMb	RELMb	Resistin	Resistin	S100b
12	TACE	TACE	TDAG51	TDAG51	TECK	TECK	TGF-a	TGF-a	TGF-b	TGF-b	Thrombospondin 1	Thrombospondin 1	Thrombospondin 2	Thrombospondin 2	Thrombospondin 4
13	TNF alpha	TNF alpha	TNFsRI	TNFsRI	TNFsRII	TNFsRII	TSG-6	TSG-6	TSH	TSH	Vaspin	Vaspin	VCAM1	VCAM1	VEGF

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	ADFP	Adiponectin / Acrp30	Adiponectin / Acrp30	Adipsin (Factor D)	Adipsin (Factor D)	AgRP	AgRP	AMPKa1	AMPKa1	Amylin	Amylin	Angiopoietin-1	Angiopoietin-1	Angiopoietin-2	Angiopoietin-2
2	ApoB	ApoE	ApoE	Axl	Axl	BDNF	BDNF	bFGF	bFGF	BMP-2	BMP-2	BMP-3	BMP-3	BMP-3b / GDF-10	BMP-3b / GDF-10
3	BMPR-IB / ALK-6	BMPR-II	BMPR-II	b-NGF	b-NGF	C3a des Arg	C3a des Arg	CART	CART	CD137 (4-1BB)	CD137 (4-1BB)	CD36	CD36	Clusterin	Clusterin
4	ENA-78	Endophin Beta	Endophin Beta	Epiregulin	Epiregulin	E-selectin	E-selectin	ET-1 (Endothelin)	ET-1 (Endothelin)	FABP4	FABP4	FAM3B	FAM3B	FAS / Apo-1	FAS / Apo-1
5	GITRL	GLP-1	GLP-1	Glucagon	Glucagon	Glut1	Glut1	Glut2	Glut2	Glut3	Glut3	Glut5	Glut5	Glutathione peroxidase 1	Glutathione peroxidase 1
6	IGF-1	IGF-1 sR	IGF-1 sR	IGFBP-1	IGFBP-1	IGFBP-2	IGFBP-2	IGFBP-3	IGFBP-3	IGF-II	IGF-II	IL-1 R1	IL-1 R1	IL-1 R4	IL-1 R4
7	IL-11	IL-12	IL-12	IL-25 / IL-17E	IL-25 / IL-17E	INSL3	INSL3	INSRR	INSRR	Insulin	Insulin	Insulin R (CD220)	Insulin R (CD220)	Leptin	Leptin
8	LOX	Lymphotactin	Lymphotactin	MCP-1	MCP-1	MCP-3	MCP-3	M-CSF	M-CSF	MIF	MIF	MIP-1a	MIP-1a	MIP-1b	MIP-1b
9	Myostatin	NAIP	NAIP	NeuroD1	NeuroD1	Neurophilin-2	Neurophilin-2	NGF R	NGF R	NPY (Neuropeptide Y)	NPY (Neuropeptide Y)	Obestatin R (GPR-39)	Obestatin R (GPR-39)	Orexin A	Orexin A
10	PDGF-AA	PDGF-AB	PDGF-AB	PDGF-C	PDGF-C	PDGF-D	PDGF-D	PEDF	PEDF	Pentraxin-3	Pentraxin-3	PPARg2 / NRIC3	PPARg2 / NRIC3	Pref-1	Pref-1
11	S100b	S100 A8+A9	S100 A8+A9	S100 A10	S100 A10	SAA	SAA	SDF-1	SDF-1	SEMA3A	SEMA3A	Serotonin	Serotonin	Syndecan-3	Syndecan-3
12	Thrombospondin 4	TIMP-1	TIMP-1	TIMP-2	TIMP-2	TIMP-3	TIMP-3	TIMP-4	TIMP-4	Tissue factor (CD142)	Tissue factor (CD142)	TLR2	TLR2	TLR4	TLR4
13	VEGF	Visfatin/PBEF1	Visfatin/PBEF1	XEDAR	XEDAR	NEG	NEG	NEG	NEG	Pos 3	Pos 3	Pos 2	Pos 2	Pos 1	Pos 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

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