RayBio® Biotin Label-based Human Antibody Array 1

For the Simultaneous Detection of the Relative Expression of 507 Human Proteins in Cell Culture Supernates, Serum or Plasma

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

User Manual (Revised Jul 18, 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

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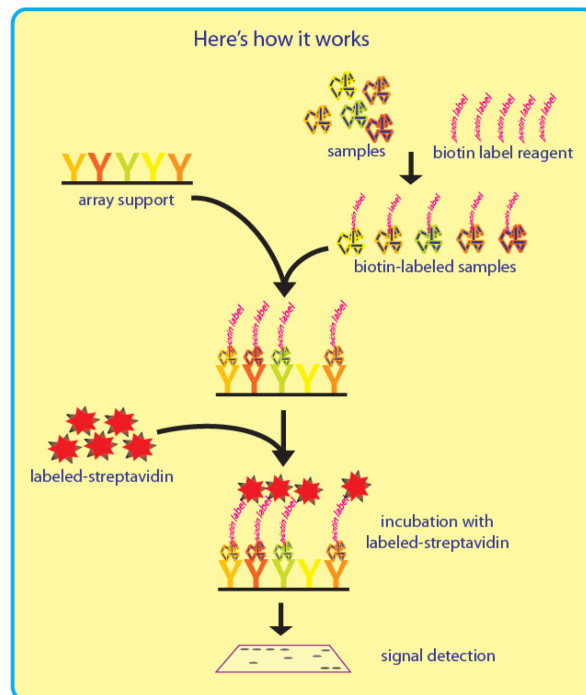
I. Introduction

Recent technological advances by Raybiotech have enabled the largest commercially available antibody array to date. With the L Series 507, researchers can now obtain a broad, panoramic view of cytokine expression. The expression levels of 507 human target 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 Human 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, which is 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 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 per glass chip)
- Labeling Reagent (Item B, 1 tube per glass chip)
- Stop Solution (Item D, 50 µl)
- RayBio® Biotin Label-based Human Antibody Array 1 Glass Chip in Chamber Assembly (Item E, 2 sub-arrays per glass chip). Kits may contain 1 glass chip for 2 samples or 2 glass chips for 4 samples
- 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

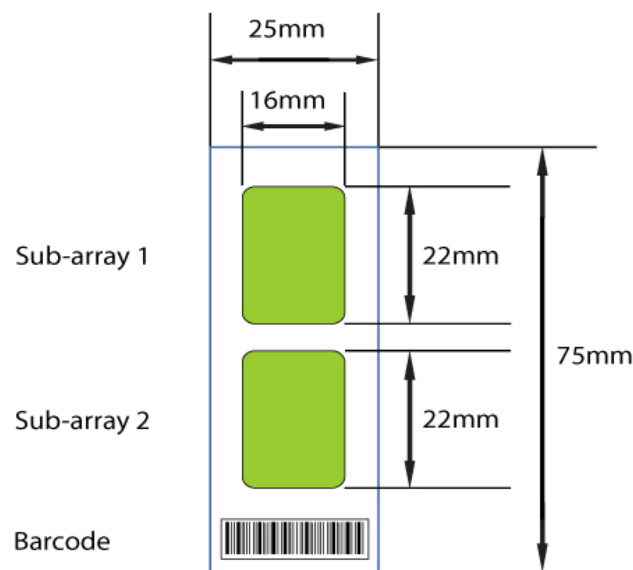
- The microarray slides are delicate. Please do not touch the array surface with pipette tips, forceps or your fingers. Hold the slides by the edges only.
- Handle the slides with powder-free gloves and in a clean environment.
- Do not remove the glass chip from the chamber assembly until step 19, and 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



2 printed sub-arrays per glass chip

D. Preparation of Cell Culture Supernates

1). Plate cells at a density of 1×10^6 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.*

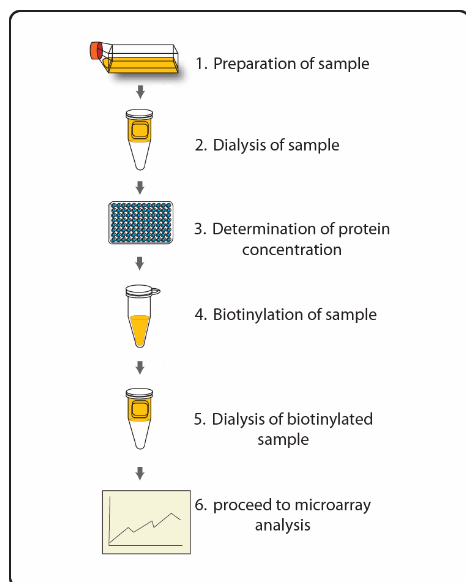
** *Optimal culture time may be different and depends on your cell lines, treatment conditions and other factors.*

† *Bovine serum proteins produce detectable signals on the RayBio® Human 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.*

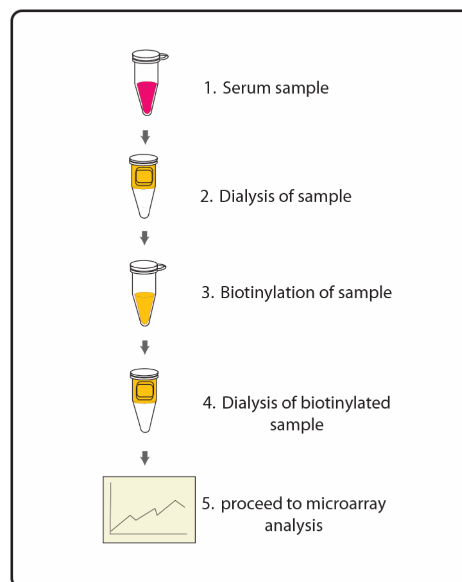
V. Protocol

Assay Diagram

1. Cell Culture Supernates



2. Serum or plasma



<u>Dialysis of Sample</u>

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 <u>not</u> 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 <u>completely</u> 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.

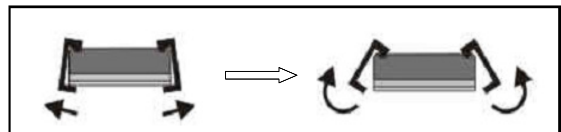
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Chip Assembly with aluminum foil or incubate in dark room.

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).***

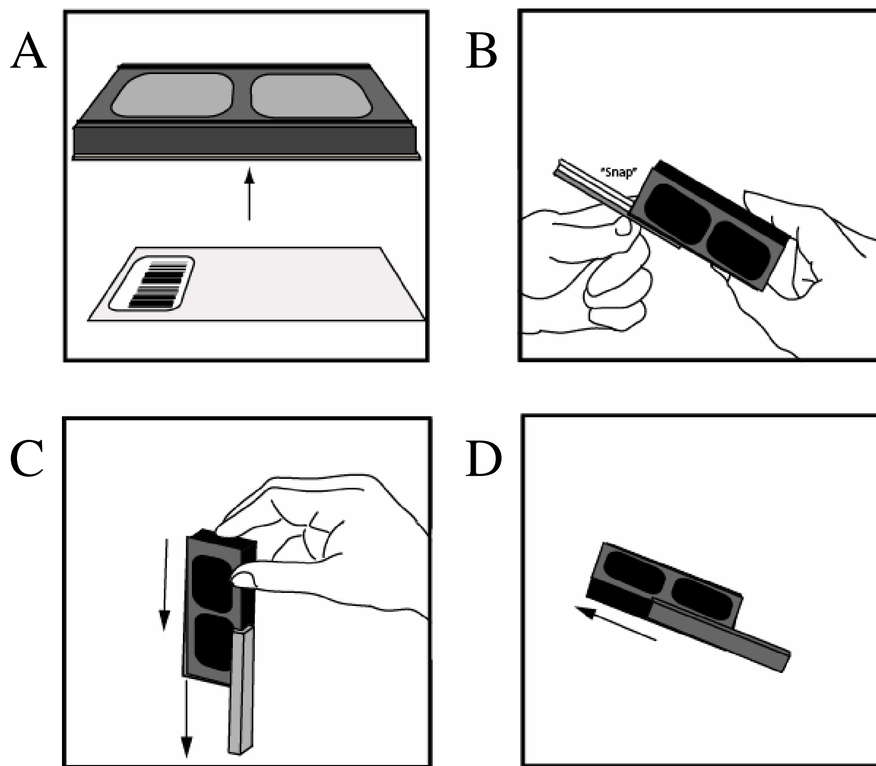
Fluorescence Detection

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:

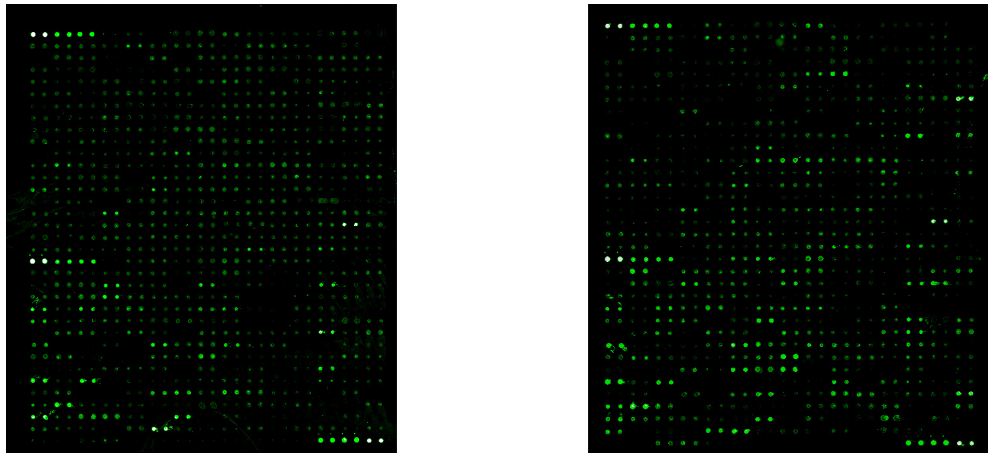
A. Explanation of Controls Spots

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 Antibody Array 1

The following figure shows the RayBio® **Biotin-label-based Human Antibody Array 1** probed with serum sample. The images were captured using a Axon GenePix laser scanner. The strong signals in row 20 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 Antibody Array 1 Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	P-1a	P-1a	P-2a	P-2a	P-3a	P-3a	Neg	Neg	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12	13	13	14	14	15	15
2	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26	27	27	28	28	29	29	30	30
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35	511	511	512	512	513	513	514	514	515	515	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	P-3c	P-3c	P-2c	P-2c	P-1c	P-1c

RayBio® Biotin Label-based Human Antibody Array 1 List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	Positive 1a	61	CCR7	121	Eotaxin-2 / MPIF-2	181	GFR alpha-2	241	IL-1 R6 / IL-1 Rrp2
2	Positive 2a	62	CCR8	122	Eotaxin-3 / CCL26	182	GFR alpha-3	242	IL-1 R8
3	Positive 3a	63	CCR9	123	Epiregulin	183	GFR alpha-4	243	IL-1 R9
4	neg	64	CD14	124	ErbB2	184	GITR / TNFRF18	244	IL-1 ra
5	6Ckine	65	CD27 / TNFRSF7	125	ErbB3	185	GITR Ligand / TNFSF18	245	IL-1 sRI
6	Activin A	66	CD30 / TNFRSF8	126	ErbB4	186	Glucagon	246	IL-1 sRII
7	Activin B	67	CD30 Ligand / TNFSF8	127	Erythropoietin	187	Glut1	247	IL-2
8	Activin C	68	CD40 / TNFRSF5	128	E-Selectin	188	Glut2	248	IL-2 R alpha
9	Activin RIA / ALK-2	69	CD40 Ligand / TNFSF5 /CD154	129	FADD	189	Glut3	249	IL-2 R beta /CD122
10	Activin RIB / ALK-4	70	CD 163	130	FAM3B	190	Glut5	250	IL-2 R gamma
11	Activin RII A/B	71	Cerberus 1	131	Fas / TNFRSF6	191	Glypican 3	251	IL-3
12	Activin RIIA	72	Chem R23	132	Fas Ligand	192	Glypican 5	252	IL-3 R alpha
13	Adiponectin / Acrp30	73	Chordin-Like 1	133	FGF Basic	193	GM-CSF	253	IL-4
14	AgRP	74	Chordin-Like 2	134	FGF-BP	194	GM-CSF R alpha	254	IL-4 R
15	ALCAM	75	CLC	135	FGF R3	195	Granzyme A	255	IL-5
16	Angiogenin	76	CNTF	136	FGF R4	196	GREMLIN	256	IL-5 R alpha
17	Angiopoietin-1	77	CNTF R alpha	137	FGF R5	197	GRO	257	IL-6
18	Angiopoietin-2	78	Coagulation Factor III / Tissue Factor	138	FGF-4	198	GRO-a	258	IL-6 R
19	Angiopoietin-4	79	CRIM 1	139	FGF-5	199	Growth Hormone (GH)	259	IL-7
20	Angiopoietin-like 1	80	Cripto-1	140	FGF-6	200	Growth Hormone R (GHR)	260	IL-7 R alpha
21	Angiopoietin-like 2	81	CRTH-2	141	FGF-7 / KGF	201	HB-EGF	261	IL-8
22	Angiopoietin-like Factor	82	Cryptic	142	FGF-8	202	HCC-4 / CCL16	262	IL-9
23	Angiostatin	83	Csk	143	FGF-9	203	HCR / CRAM-A/B	263	IL-10
24	APJ	84	CTACK / CCL27	144	FGF-10 / KGF-2	204	Hepassocin	264	IL-10 R alpha
25	APRIL	85	CTGF / CCN2	145	FGF-11	205	Heregulin / NDF / GGF	265	IL-10 R beta
26	AR (Amphiregulin)	86	CTLA-4 /CD152	146	FGF-12	206	HGF	266	IL-11
27	Artemin	87	CV-2 / Crossveinless-2	147	FGF-13 1B	207	HGFR	267	IL-12 p40
28	Axl	88	CXCL14 / BRAK	148	FGF-16	208	HRG-alpha	268	IL-12 p70
29	B7-1 /CD80	89	CXCL16	149	FGF-17	209	HRG-beta 1	269	IL-12 R beta 1
30	BAFF R / TNFRSF13C	90	CXCR1 / IL-8 RA	150	FGF-18	210	HVEM / TNFRSF14	270	IL-12 R beta 2
31	BCMA / TNFRSF17	91	CXCR2 / IL-8 RB	151	FGF-19	211	I-309	271	IL-13
32	BD-1	92	CXCR3	152	FGF-20	212	ICAM-1	272	IL-13 R alpha 1
33	BDNF	93	CXCR4 (fusin)	153	FGF-21	213	ICAM-2	273	IL-13 R alpha 2
34	beta-Catenin	94	CXCR5 /BLR-1	154	FGF-23	214	ICAM-3 (CD50)	274	IL-15
35	beta-Defensin 2	95	CXCR6	155	FLRG	215	ICAM-5	275	IL-15 R alpha
36	beta-NGF	96	D6	156	Flt-3 Ligand	216	IFN-alpha / beta R1	276	IL-16
37	BIK	97	DAN	157	Follistatin	217	IFN-alpha / beta R2	277	IL-17
38	BLC / BCA-1 / CXCL13	98	DANCE	158	Follistatin-like 1	218	IFN-beta	278	IL-17B
39	BMP-2	99	DcR3 / TNFRSF6B	159	Fractalkine	219	IFN-gamma	279	IL-17B R
40	BMP-3	100	Decorin	160	Frizzled-1	220	IFN-gamma R1	280	IL-17C
41	BMP-3b / GDF-10	101	Dkk-1	161	Frizzled-3	221	IGFBP-1	281	IL-17D
42	BMP-4	102	Dkk-3	162	Frizzled-4	222	IGFBP-2	282	IL-17E
43	BMP-5	103	Dkk-4	163	Frizzled-5	223	IGFBP-3	283	IL-17F
44	BMP-6	104	DR3 / TNFRSF25	164	Frizzled-6	224	IGFBP-4	284	IL-17R
45	BMP-7	105	DR6 / TNFRSF21	165	Frizzled-7	225	IGFBP-6	285	IL-17RC
46	BMP-8	106	Dtk	166	Galectin-3	226	IGFBP-rp1 / IGFBP-7	286	Positive 1b
47	BMP-15	107	EDA-A2	167	GASP-1 / WFIKKNRP	227	IGF-I	287	Positive 2b
48	BMPR-IA / ALK-3	108	EDAR	168	GASP-2 / WFIKKN	228	IGF-I SR	288	Positive 3b
49	BMPR-IB / ALK-6	109	EDG-1	169	GCP-2 / CXCL6	229	IGF-II	289	neg
50	BMPR-II	110	EGF	170	GCSF	230	IGF-II R	290	IL-17RD
51	BTC	111	EGF R / ErbB1	171	G-CSF R / CD 114	231	IL-1 alpha	291	IL-18 BPa
52	Cardiotrophin-1 / CT-1	112	EG-VEGF / PK1	172	GDF1	232	IL-1 beta	292	IL-18 R alpha /IL-1 R5
53	CCL14 / HCC-1 / HCC-3	113	EMAP-II	173	GDF3	233	IL-1 F5 / FIL1delta	293	IL-18 R beta /AcPL
54	CCL28 / VIC	114	ENA-78	174	GDF5	234	IL-1 F6 / FIL1 epsilon	294	IL-19
55	CCR1	115	Endocan	175	GDF8	235	IL-1 F7 / FIL1 zeta	295	IL-20
56	CCR2	116	Endoglin / CD105	176	GDF9	236	IL-1 F8 / FIL1 eta	296	IL-20 R alpha
57	CCR3	117	Endostatin	177	GDF11	237	IL-1 F9 / IL-1 H1	297	IL-20 R beta
58	CCR4	118	Endothelin	178	GDF-15	238	IL-1 F10 / IL-1HY2	298	IL-21
59	CCR5	119	EN-RAGE	179	GDNF	239	IL-1 R3 / IL-1 R AcP	299	IL-21 R
60	CCR6	120	Eotaxin / CCL11	180	GFR alpha-1	240	IL-1 R4 / ST2	300	IL-22

RayBio® Biotin Label-based Human Antibody Array 1 List ...continued

Number	Name	Number	Name	Number	Name	Number	Name
301	IL-22 BP	361	MMP-2	421	RANK / TNFRSF11A	481	TMEFF1 / Tomoregulin-1
302	IL-22 R	362	MMP-3	422	RANTES	482	TMEFF2
303	IL-23	363	MMP-7	423	RELM beta	483	TNF-alpha
304	IL-23 R	364	MMP-8	424	RELT / TNFRSF19L	484	TNF-beta
305	IL-24	365	MMP-9	425	ROBO4	485	TNF RI / TNFRSF1A
306	IL-26	366	MMP-10	426	S100 A8/A9	486	TNF RII / TNFRSF1B
307	IL-27	367	MMP-11 /Stromelysin-3	427	S100A10	487	TRADD
308	IL-28A	368	MMP-12	428	SAA	488	TRAIL / TNFSF10
309	IL-29	369	MMP-13	429	SCF	489	TRAIL R1 / DR4 / TNFRSF10A
310	IL-31	370	MMP-14	430	SCF R /CD117	490	TRAIL R2 / DR5 / TNFRSF10B
311	IL-31 RA	371	MMP-15	431	SDF-1 / CXCL12	491	TRAIL R3 / TNFRSF10C
312	Inhibin A	372	MMP-16 / MT3-MMP	432	sFRP-1	492	TRAIL R4 / TNFRSF10D
313	Inhibin B	373	MMP-19	433	sFRP-3	493	TRANCE
314	Insulin	374	MMP-20	434	sFRP-4	494	TREM-1
315	Insulin R	375	MMP-24 / MT5-MMP	435	sgp130	495	TROY / TNFRSF19
316	Insulysin / IDE	376	MMP-25 / MT6-MMP	436	SIGIRR	496	TSG-6
317	IP-10	377	MSP alpha Chain	437	Siglec-5/CD170	497	TSLP R
318	I-TAC / CXCL11	378	Musk	438	Siglec-9	498	TWEAK / TNFSF12
319	Kininostatin / kininogen	379	NAP-2	439	SLPI	499	TWEAK R / TNFRSF12
320	Kremen-1	380	NCAM-1 / CD56	440	Smad 1	500	Ubiquitin+1
321	Kremen-2	381	Neuritin	441	Smad 4	501	uPA
322	Latent TGF-beta bp1	382	NeuroD1	442	Smad 5	502	uPAR
323	LBP	383	Neuropilin-2	443	Smad 7	503	Vasorin
324	Lck	384	Neurturin	444	Smad 8	504	VCAM-1 (CD106)
325	LECT2	385	NGF R	445	SMDF / NRG1Isoform	505	VE-Cadherin
326	Lefty - A	386	Nidgen-1	446	Soggy-1	506	VEGF
327	Leptin (OB)	387	NOV / CCN3	447	Sonic Hedgehog (Shh N-terminal)	507	VEGF R2 (KDR)
328	Leptin R	388	NrCAM	448	SPARC	508	VEGF R3
329	LFA-1 alpha	389	NRG1 Isoform GGF2	449	Spinesin	509	VEGF-B
330	LIF	390	NRG2	450	TACI / TNFRSF13B	510	VEGF-C
331	LIF R alpha	391	NRG3	451	Tarc	511	VEGF-D
332	LIGHT / TNFSF14	392	NT-3	452	TCCR / WSX-1	512	VEGI / TNFSF15
333	Lipocalin-1	393	NT-4	453	TECK / CCL25	513	WIF-1
334	Lipocalin-2	394	Orexin A	454	TFPI	514	WISP-1 / CCN4
335	LRP-1	395	Orexin B	455	TGF-alpha	515	XEDAR
336	LRP-6	396	OSM	456	TGF-beta 1	516	Neg
337	L-Selectin (CD62L)	397	Osteoactivin / GPNMB	457	TGF-beta 2	517	Neg
338	Lymphotactin / XCL1	398	Osteocrin	458	TGF-beta 3	518	Neg
339	Lymphotoxin beta / TNFSF3	399	Osteoprotegerin / TNFRSF11B	459	TGF-beta 5	519	Neg
340	Lymphotoxin beta R / TNFRSF3	400	OX40 Ligand / TNFSF4	460	TGF-beta RI / ALK-5	520	Neg
341	MAC-1	401	PARC / CCL18	461	TGF-beta RII	521	Neg
342	MCP-1	402	PD-ECGF	462	TGF-beta RIIb	522	Neg
343	MCP-2	403	PDGF R alpha	463	TGF-beta RIII	523	P-3c
344	MCP-3	404	PDGF R beta	464	Thrombopoietin (TPO)	524	P-2c
345	MCP-4 / CCL13	405	PDGF-AA	465	Thrombospondin (TSP)	525	P-1c
346	M-CSF	406	PDGF-AB	466	Thrombospondin-1		
347	M-CSF R	407	PDGF-BB	467	Thrombospondin-2		
348	MDC	408	PDGF-C	468	Thrombospondin-4		
349	MFG-E8	409	PDGF-D	469	Thymopoietin		
350	MFRP	410	PECAM-1 /CD31	470	Tie-1		
351	MICA	411	Pentraxin3 / TSG-14	471	Tie-2		
352	MIF	412	Persephin	472	TIMP-1		
353	MIG	413	PF4 / CXCL4	473	TIMP-2		
354	MIP-1a	414	PIGF	474	TIMP-3		
355	MIP-1b	415	PLUNC	475	TIMP-4		
356	MIP-1d	416	Pref-1	476	TL1A / TNFSF15		
357	MIP 2	417	Progranulin	477	TLR1		
358	MIP-3 alpha	418	Prolactin	478	TLR2		
359	MIP-3 beta	419	P-selectin	479	TLR3		
360	MMP-1	420	RAGE	480	TLR4		

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

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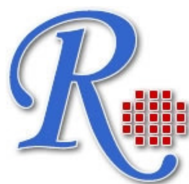

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