

RayBio[®] Biotin Label-based Mouse Antibody Array 1

**For the Simultaneous Detection of the Relative Expression of 308
Mouse Proteins in Cell Culture Supernates, Serum or Plasma**

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

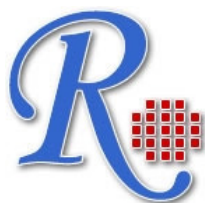
User Manual (Revised Sept 5, 2011)

**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

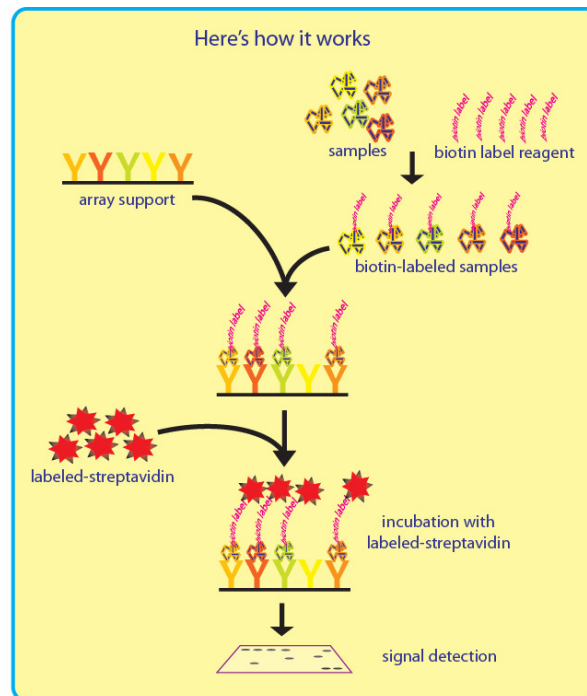
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I. Introduction

Recent technological advances by Raybiotech have enabled the largest commercially available antibody array to date. With the L Series 308, researchers can now obtain a broad, panoramic view of cytokine expression. The expression levels of 308 Mouse 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 Mouse 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 Mouse 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, KH₂PO₄ and Na₂HPO₄
- 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.

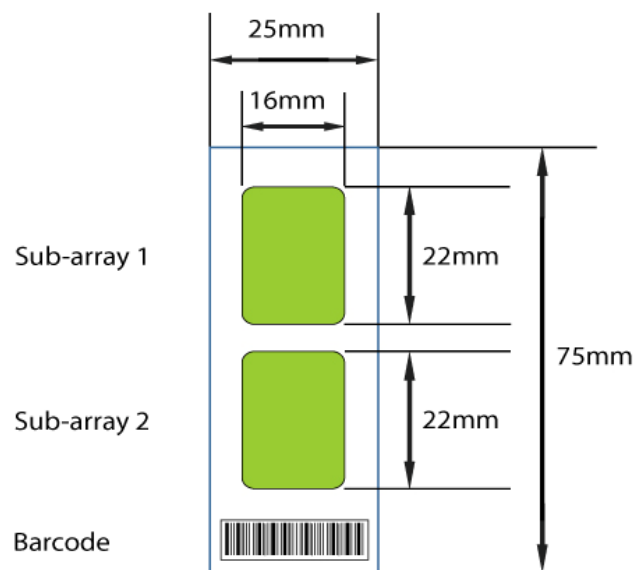


B. Incubation of Antibody Array

- 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 normalize between array by determining cell lysate 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.*

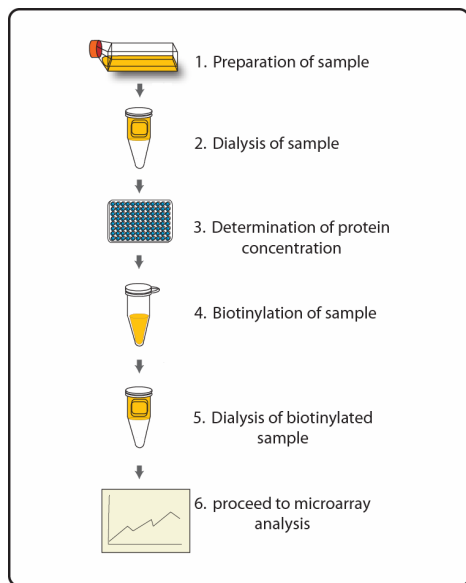
*** Optimal culture time may be different and depends on your cell lines, treatment conditions and other factors.*

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

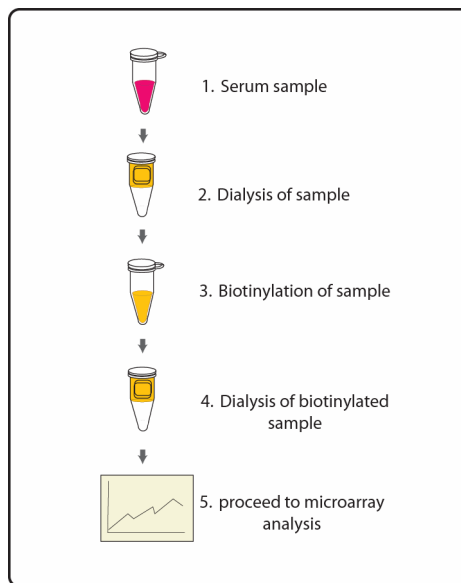
V. Protocol

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 μ l 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 μ l dialyzed sample into a new tube. Add 36 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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 μ l of Streptavidin-Fluor concentrate into a tube with 800 μ l of Blocking Buffer. Mix gently to prepare 1X working dilution.

17. Carefully remove Glass Chip Assembly from container. Remove all of Wash Buffer II from the wells. Add 400 μ l of 1X Streptavidin-conjugated Fluorescent dye to each sub-array. Cover the incubation chamber with adhesive film.

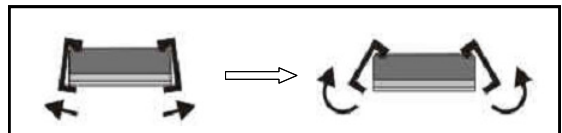
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Chip Assembly with aluminum foil or incubate in dark room.

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).

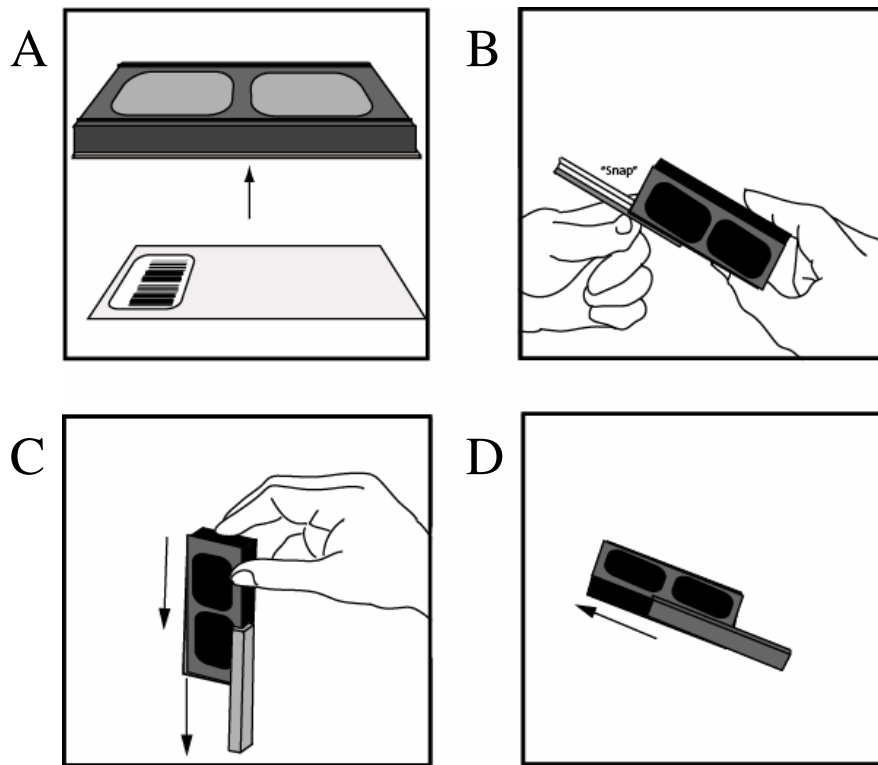
Fluorescence Detection

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).*



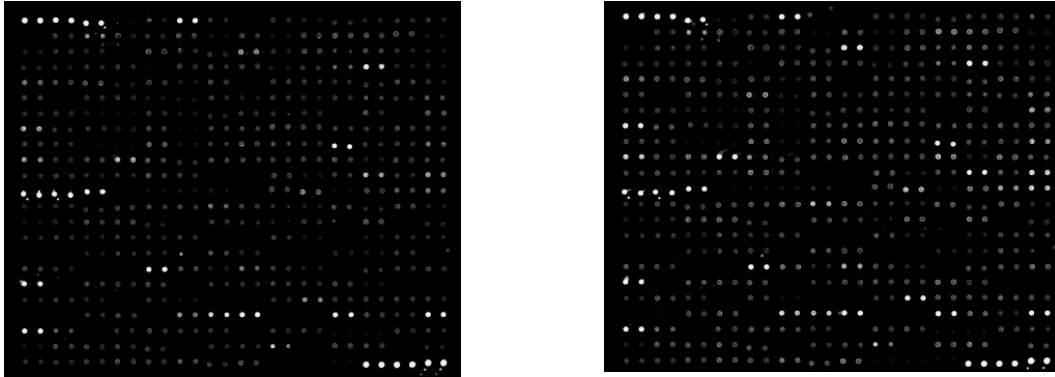
VI. Interpretation of Results:

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 Mouse Antibody Array 1

The following figure shows the RayBio[®] **Biotin-label-based Mouse Antibody Array 1** probed with serum sample. The images were captured using a Axon GenePix laser scanner. The strong signals in row 12 and 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 $\approx 95\%$).

RayBio® Biotin Label-based Mouse 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
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
2	15	15	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
3	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41	42	42
4	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56
5	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70
6	71	71	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84
7	85	85	86	86	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98
8	99	99	100	100	101	101	102	102	103	103	104	104	105	105	106	106	107	107	108	108	109	109	110	110	111	111	112	112
9	113	113	114	114	115	115	116	116	117	117	118	118	119	119	120	120	121	121	122	122	123	123	124	124	125	125	126	126
10	127	127	128	128	129	129	130	130	131	131	132	132	133	133	134	134	135	135	136	136	137	137	138	138	139	139	140	140
11	141	141	142	142	143	143	144	144	145	145	146	146	147	147	148	148	149	149	150	150	151	151	152	152	153	153	154	154
12	P-1b	P-1b	P-2b	P-2b	P-3b	P-3b	Neg	Neg	159	159	160	160	161	161	162	162	163	163	164	164	165	165	166	166	167	167	168	168
13	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176	177	177	178	178	179	179	180	180	181	181	182	182
14	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191	192	192	193	193	194	194	195	195	196	196
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16	211	211	212	212	213	213	214	214	215	215	216	216	217	217	218	218	219	219	220	220	221	221	222	222	223	223	224	224
17	225	225	226	226	227	227	228	228	229	229	230	230	231	231	232	232	233	233	234	234	235	235	236	236	237	237	238	238
18	239	239	240	240	241	241	242	242	243	243	244	244	245	245	246	246	247	247	248	248	249	249	250	250	251	251	252	252
19	253	253	254	254	255	255	256	256	257	257	258	258	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266
20	267	267	268	268	269	269	270	270	271	271	272	272	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280
21	281	281	282	282	283	283	284	284	285	285	286	286	287	287	288	288	289	289	290	290	291	291	292	292	293	293	294	294
22	295	295	296	296	297	297	298	298	299	299	300	300	301	301	302	302	303	303	304	304	305	305	306	306	307	307	308	308
23	309	309	310	310	311	311	312	312	313	313	314	314	315	315	316	316	Neg	Neg	Neg	Neg	Neg	Neg	P-3c	P-3c	P-2c	P-2c	P-1c	P-1c

RayBio® Biotin Label-based Mouse Antibody Array 1 List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	Positive 1a	57	CXCL16	113	Granzyme D	169	IL-12 R beta 1	225	MIP-2	281	TIMP-2
2	Positive 2a	58	CXCR2 / IL-8 RB	114	Granzyme G	170	IL-13	226	MIP-3 alpha	282	TIMP-4
3	Positive 3a	59	CXCR3	115	Gremlin	171	IL-13 R alpha 2	227	MIP-3 beta	283	TL1A / TNFSF15
4	neg	60	CXCR4	116	Growth Hormone R	172	IL-15	228	MMP-2	284	TLR1
5	6Ckine	61	CXCR6	117	HGF R	173	IL-15 R alpha	229	MMP-3	285	TLR2
6	Activin A	62	DAN	118	HGF	174	IL-16	230	MMP-9	286	TLR3
7	Activin C	63	Decorin	119	HVEM / TNFRSF14	175	IL-17	231	MMP-12	287	TLR4
8	Activin RIB / ALK-4	64	DKK-1	120	ICAM-1	176	IL-17BR	232	MMP-14 / LEM-2	288	TMEFF1 / Tomoregulin-1
9	Adiponectin / Acrp30	65	Dkk-3	121	ICAM-2 / CD102	177	IL-17C	233	MMP-24 / MTS-MMP	289	TNF RI / TNFRSF1A
10	AgRP	66	Dkk-4	122	ICAM-5	178	IL-17D	234	Neuregulin-3 / NRG3	290	TNF RII
11	ALCAM	67	DPPIV / CD26	123	ICK	179	IL-17E	235	Neurturin	291	TNF-alpha
12	Angiopoietin-like 2	68	DR3 / TNFRSF25	124	IFN-alpha / beta R1	180	IL-17F	236	NGF R / TNFRSF16	292	TNF-beta / TNFSF1B
13	Angiopoietin-like 3	69	Dtk	125	IFN-alpha / beta R2	181	IL-17R	237	NOV / CCN3	293	TPO
14	AR (Amphiregulin)	70	EDAR	126	IFN-beta	182	IL-17RC	238	Osteoactivin / GPNMB	294	TRAIL / TNFSF10
15	Artemin	71	EGF R	127	IFN-gamma	183	IL-17RD	239	Osteopontin	295	TRAIL R2 / TNFRSF10B
16	Axl	72	EG-VEGF / PK1	128	IFN-gamma R1	184	IL-18 R alpha/IL-1 R5	240	Osteoprotegerin	296	TRANCE / TNFSF11
17	b FGF	73	Endocan	129	IGFBP-1	185	IL-20	241	OX40 Ligand / TNFSF4	297	TREM-1
18	B7-1/CD80	74	Endoglin / CD105	130	IGFBP-2	186	IL-20 R alpha	242	PDGF C	298	TROY
19	BAFF R / TNFRSF13C	75	Endostatin	131	IGFBP-3	187	IL-21	243	PDGF R alpha	299	TSLP
20	BCMA / TNFRSF17	76	Eotaxin	132	IGFBP-5	188	IL-21 R	244	PDGF R beta	300	TSLP R
21	beta-Catenin	77	Eotaxin-2	133	IGFBP-6	189	IL-22	245	Pentraxin3 / TSG-14	301	TWEAK / TNFSF12
22	BLC	78	Epigen	134	IGFBP-rp1 / IGFBP-7	190	IL-22BP	246	PF-4	302	TWEAK R / TNFRSF12
23	BTC (Betacellulin)	79	Epregrulin	135	IGF-I	191	IL-23	247	PIGF-2	303	Ubiquitin
24	Cardiotrophin-1	80	Erythropoietin (EPO)	136	IGF-II	192	IL-23 R	248	Progranulin	304	uPAR
25	CCL1 / I-309 / TCA-3	81	E-Selectin	137	IL-1 alpha	193	IL-24	249	Prolactin	305	Urokinase
26	CCL28	82	FADD	138	IL-1 beta	194	IL-27	250	P-Selectin	306	VCAM-1
27	CCL4 / MIP-1 beta	83	FAM3B	139	IL-1 R4 / ST2	195	IL-28 / IFN-lambda	251	RAGE	307	VE-Cadherin
28	CCL7 / MCP-3 / MARC	84	Fas / TNFRSF6	140	IL-1 R6 / IL-1 R p2	196	IL-31	252	RANTES	308	VEGF
29	CCL8 / MCP-2	85	Fas Ligand	141	IL-1 R9	197	IL-31 RA	253	RELM beta	309	VEGF R1
30	CCR10	86	FCγRIIB / CD32b	142	IL-1 RI	198	Insulin	254	Resistin	310	VEGF R2
31	CCR3	87	FGF R3	143	IL-1 RII	199	Integrin beta 2 / CD18	255	SI00A10	311	VEGF R3
32	CCR4	88	FGF R4	144	IL-2	200	I-TAC	256	SCF	312	VEGF-B
33	CCR6	89	FGF R5 beta	145	IL-2 R alpha	201	KC	257	SCF R / c-kit	313	VEGFC
34	CCR7	90	FGF-21	146	IL-2 R beta	202	Kremen-1	258	SDF-1	314	VEGF-D
35	CCR9	91	Fit-3 Ligand	147	IL-3	203	Kremen-2	259	Serum Amyloid A1	315	WIF-1
36	CD11b	92	FLRG (Follistatin)	148	IL-3 R alpha	204	Lefty-1	260	Shh-N	316	WISP-1 / CCN4
37	CD14	93	Follistatin-like 1	149	IL-3 R beta	205	Leptin R	261	SIGIRR	317	Neg
38	CRP	94	Fractalkine	150	IL-4	206	LEPTIN(OB)	262	SLPI	318	Neg
39	CD27 / TNFRSF7	95	Frizzled-1	151	IL-4 R	207	LIF	263	Soggy-1	319	Neg
40	CD27 Ligand / TNFSF7	96	Frizzled-6	152	IL-5	208	LIGHT / TNFSF14	264	SPARC	320	Positive 3c
41	CD30	97	Frizzled-7	153	IL-5 R alpha	209	LIX	265	Spinesin Ectodomain	321	Positive 2c
42	CD30 L	98	Galectin-3	154	IL-6	210	LRP-6	266	TACI / TNFRSF13B	322	Positive 1c
43	CD40	99	G-CSF	155	Positive 1b	211	L-Selectin	267	TARC	323	
44	CD40 Ligand / TNFSF5	100	GDF-1	156	Positive 2b	212	Lungkine	268	TCA-3	324	
45	Cerberus 1	101	GDF-3	157	Positive 3b	213	Lymphotoxin	269	TCCR / WSX-1	325	
46	Chordin-Like 2	102	GDF-5	158	neg	214	lymphotoxin beta R / TNFRSF	270	TECK	326	
47	Regulation Factor III / Tissue Factor	103	GDF-8	159	IL-6 R	215	MAdCAM-1	271	TFPI	327	
48	mon gamma Chain / IL-2 R gamma	104	GDF-9	160	IL-7	216	MCP-1	272	TGF-beta 1	328	
49	CRG-2	105	GFR alpha-2 / GDNF R alpha-2	161	IL-7 R alpha	217	MCP-5	273	TGF-beta 2	329	
50	Cripto	106	GFR alpha-3 / GDNF R alpha-3	162	IL-9	218	M-CSF	274	TGF-beta 3	330	
51	Crossveinless-2	107	GFR alpha-4 / GDNF R alpha-4	163	IL-9 R	219	MDC	275	TGF-beta RI / ALK-5	331	
52	Cryptic	108	GITR	164	IL-10	220	MFG-E8	276	TGF-beta RII	332	
53	Csk	109	GITR Ligand / TNFSF18	165	IL-10 R alpha	221	MFRP	277	Thrombospondin	333	
54	CTACK	110	Glut2	166	IL-11	222	MIG	278	Thymus Chemokine-1	334	
55	CTLA-4 / CD152	111	GM-CSF	167	IL-12 p40/p70	223	MIP-1 alpha	279	Tie-2	335	
56	CXCL14 / BRAK	112	Granzyme B	168	IL-12 p70	224	MIP-1 gamma	280	TIMP-1	336	

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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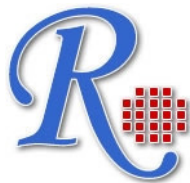
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