

RayBio® Label-Based (L-Series)

Mouse Antibody Array L-2808 Glass Slide Kit

A combination of Mouse L-308, L-2, L-3, L-4, L-5, and L-6 arrays

Patent Pending Technology

User Manual (February 1, 2024)

For the simultaneous detection of the relative expression of 2808 mouse proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Mouse Antibody Array L-2808
Cat# AAM-BLG-2808-4 (4 Sample Kit)
Cat# AAM-BLG-2808-8 (8 Sample Kit)

**Please read manual carefully
before starting experiment**



Your Provider of Excellent Protein Array Systems and Services

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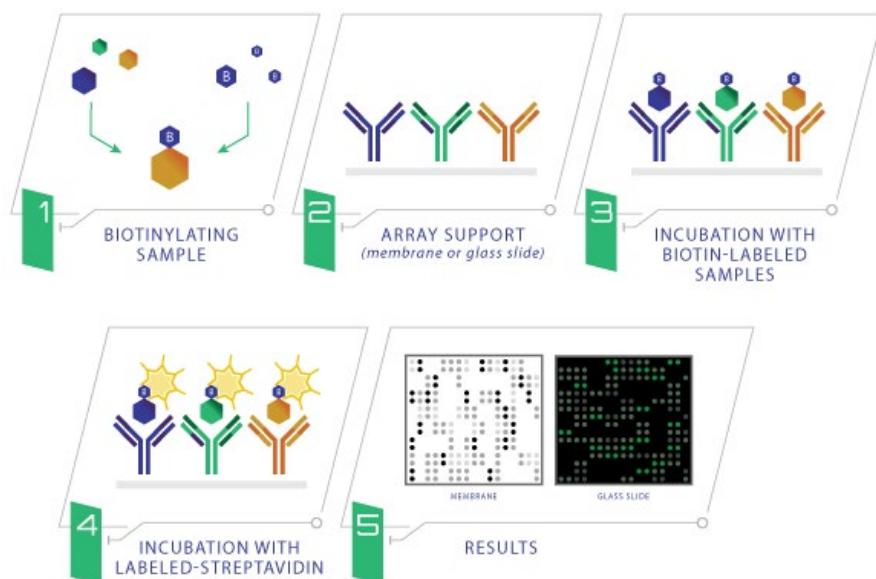
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I. Introduction

Combining direct antigen-labeling technology with our vast library of array-validated antibodies, RayBiotech has created the largest commercially available antibody array to date. With the L-Series high density array platform, researchers can now detect thousands of proteins simultaneously, obtaining a broad, panoramic view of protein expression. Our newly expanded panel includes a wide variety of metabolic enzymes, structural proteins, epigenetic markers, neuroregulatory factors, in addition to our popular list of cytokines, growth factors, receptors, adipokines, proteases, and signaling proteins. Available on both glass slide and membrane formats, this array is ideally suited for biomarker discovery studies and exploratory screens.

The first step in using the RayBio® L-Series Antibody Array is to biotinylate the primary amine groups of the proteins in your sample (sera or plasma, cell culture supernatants, cell lysates or tissue lysates). The glass slide arrays are then blocked, just like a western blot, and the biotin-labeled sample is added onto the glass slide, which is pre-printed with capture antibodies. The slide is incubated to allow binding of target proteins. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then applied to the array. Finally, the glass slide is dried, and laser fluorescence scanning is used to visualize the signals.



II. Materials Provided

A. Storage Recommendations

Upon receipt, the kit should be stored at -20°C until needed. It is recommended to use the kit within 6 months of the date of shipment. After initial use, remaining reagents should be stored at 4°C and may be stored for up to 3 months. Labeling Reagent (Item B) should be prepared fresh each time before use. Unused glass slides should be kept at -20 °C and repeated freeze-thaw cycles should be avoided (slides may be stored for 6 months).

ITEM	DESCRIPTION	4 SAMPLE Kit	8 SAMPLE Kit
A	Spin Columns (0.5ml)	16 columns	32 columns
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	2 vials (50 µl)
E	RayBio® L-Series Glass Slide*	1 slide each of Mouse L-308, L-2, L-3, L-4, L-5, and L-6	2 slides each of Mouse L-308, L-2, L-3, L-4, L-5, and L-6
F	Blocking Buffer	2 bottles (30 ml)	3 bottles (30 ml)
G	20X Wash Buffer I	2 bottles (30 ml)	3 bottles (30 ml)
H	20X Wash Buffer II	2 bottles (30 ml)	3 bottles (30 ml)
I	Cy3 equivalent-Conjugated Streptavidin	3 vials	5 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (30 ml)	2 bottles (30 ml)
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	2 bottles (10 ml)
M	30 ml Centrifuge Tube	2 tubes	3 tubes

*Each slide contains 4 identical subarrays

**Only needed if testing cell or tissue lysates

B. Additional Materials Required

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

III. Overview and General Considerations

A. Preparation and Storage of Samples

1) Preparation of Cell Culture Supernatants

1. Seed cells at a density of 1×10^6 cells in 100 mm tissue culture dishes.*
2. Culture cells 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.**,† The membrane-based array is recommended if high serum medium such as 10% FCS/FBS is used, as high background can occur on glass slide arrays with high serum containing media samples.
4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as ≤ 1 ml aliquots at -80°C until needed.
5. If you want to use cell mass for inter-sample normalization, 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 arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

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

***Optimal culture time may vary and will depend on the cell line, treatment conditions and other factors.*

†Bovine serum proteins produce detectable signals on the RayBio® L-Series 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.

2) Extracting Protein from Cells

1. Centrifuging Cells

a. Adherent Cells:

- i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.
- ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.

b. Cells in Suspension: Pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 minutes.

2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH₂O). Solubilize the cells at 2x10⁷ cells/ml in 1X Cell Lysis Buffer.
3. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8 °C.

Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer lysates to a clean tube. Determining cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.

3) Extracting Protein from Crude Tissue

1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH₂O).

2. Homogenize the tissue according to homogenizer manufacturer instructions.
3. Transfer extracts to microcentrifuge tubes and centrifuge for 20 minutes at 13,000 rpm (4°C).

Note: If the supernatant appears to be cloudy, transfer the supernatants to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the supernatant is still not clear, store the lysate at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer supernatant to a clean tube and store at -80°C.
- 4) Determine the total protein concentration
For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

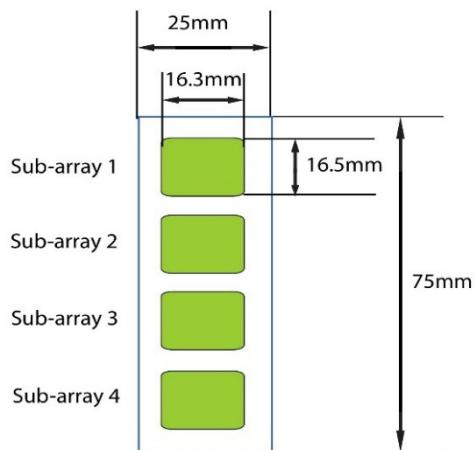
B. Handling the Glass Slides

- 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 slide from the chamber assembly until step 20, and take great care not to break the glass slide when doing so.
- Remove reagents/sample by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides as seen in image below.



C. Layout of Array Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

D. Incubations and Washes

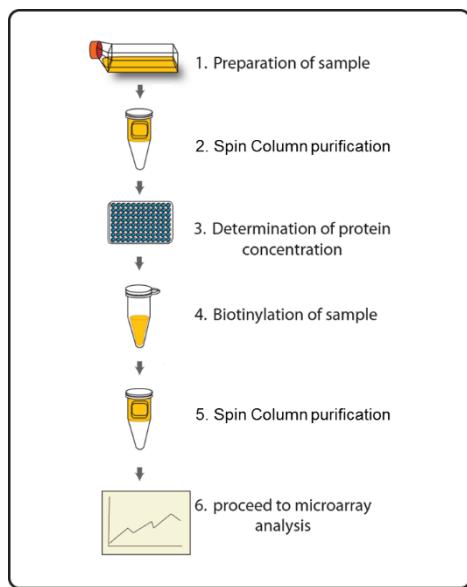
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.

- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec).
- 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 Slide Assembly to decant, and aspirate the remaining liquid.
- Unlike most Cy3 fluors, the streptavidin-conjugated fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from direct, strong light and temperatures above RT.

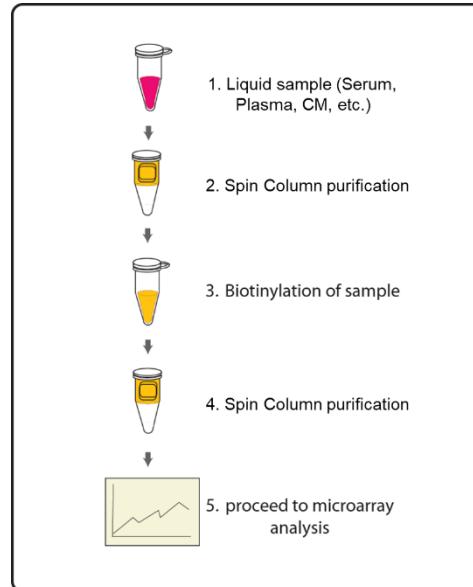
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



2. Serum, plasma, body fluid, or Cell culture supernatants



A. Sample purification

Note: This step removes low molecular weight amine derivatives or unwanted buffer from samples to ensure quality biotinylation in Steps 5–7.

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).
2. Place the Spin column into a collection tube, centrifuge at 1,500 × g for 1 minute to remove the storage buffer. Discard the flow-through.
3. Wash the Spin Column three times with 300 µl Labeling Buffer each, centrifuge at 1,500 × g for 1 minute to remove the flow-through. Discard the flow-through and blot the bottom of the column to

remove excess liquid. Transfer the Spin Column to a new collection tube.

4. Apply sample on top of the resin within the next few minutes. Centrifuge at 1,500 x g for 2 minutes. Collect the flow-through that contains the sample. The recommended sample dilutions are as follows:

- *Culture Media: 120 µl neat supernatant*
- *Serum/Plasma: 2 µl serum/plasma in 100 µl labeling buffer*
- *Cell/tissue lysate: 20 µg lysate in 100 µl labeling buffer*

Note: Each labelled sample volume is enough for at least 3 arrays following the protocol below.

Note: The maximal sample volume is 130 µl for each Spin Column. Do not load over 130 µl of sample into a Spin Column.

B. Biotin-Labeling the Sample

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

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.
6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.
 - a. For labeling cell culture supernatants: Add 8 µl of Labeling Reagent into the sample tube (for 120 µl supernatant).

- b. For labeling serum or plasma: Add 8 µl of Labeling Reagent Solution into the sample tube (for 2 µl serum/plasma *in 100 µl labeling buffer*).
- c. For labeling cell or tissue lysates: Add 4 µl of Labeling Reagent Solution into the sample tube (for 20 µg lysate *in 100 µl labeling buffer*).
- d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If the amount of sample being labelled differs from the example in Step 6, adjust this volume proportionally.

7. Add 3 µl Stop Solution (Item D) to each sample tube. Using a new spin column, repeat Steps 1-4 of section A. Sample Purification to remove the excess non-reacted biotin reagent from each sample.

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

C. Drying the Glass Slide

8. Remove the package containing the Assembled Glass Slide (Item E) from the freezer. Place unopened package on the bench top for ~15 minutes, and allow the Assembled Glass Slide to equilibrate to RT.
9. Open package, and take the Assembled Glass Slide out of the sleeve. Do not disassemble the Glass Slide from the chamber assembly. Place glass slide assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubations

Note: Glass slide 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 Assembled Glass Slide and incubating at RT for 30 minutes. Ensure there are no bubbles on the array surfaces.
11. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 10-fold for cell culture supernatants, 20-fold for serum/plasma and 100-fold for cell/tissue lysate. *Dilution for other body fluid needs to be determined by the end user. Generally, most samples can be 10-20x dilution, while tears and saliva samples may need 100x dilution.*

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.

12. Completely remove the Blocking Buffer from each well. Add 400 µl of diluted sample 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: Avoid the flow of sample into neighboring wells.

13. Based on number of samples and remaining protocol, calculate the amount of 1X Wash Buffer I and 1X Wash Buffer II needed to complete the experiment. Separately dilute the required amounts of 20X Wash Buffer I Concentrate (Item G) 20-fold and 20X Wash Buffer II Concentrate (Item H) with ddH₂O.
14. 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 minutes per wash.

15. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer I to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 10 minutes per wash.
16. Decant the Wash Buffer I from each well, place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer II to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 5 minutes per wash.
17. Prepare 1X Cy3-Conjugated Streptavidin:
 - a) Briefly spin down tube containing the Cy3-Conjugated Streptavidin (Item I) immediately before use.
 - b) Add 1000 µl of Blocking Buffer into the Cy3-Conjugated Streptavidin tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).
 - c) To prepare 1X Cy3-Conjugated Streptavidin, add 200 µl of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800 µl of Blocking Buffer. Mix gently.
18. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400 µl of 1X Cy3-Conjugated Streptavidin to each sub-array. Cover the incubation chamber with the plastic adhesive strips.

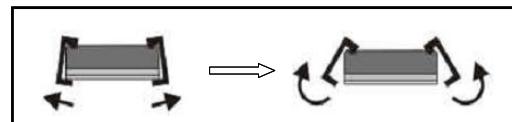
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 1 hour with gentle rocking or shaking.

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

20. Decant the solution and disassemble the glass slide from the incubation frame and chamber. Disassemble the device by pushing clips outward from the side, as shown below. Carefully remove the glass slide from the gasket.

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



21. Gently place the glass slide into 30 ml Centrifuge Tube (Item M). Add enough 1X Wash Buffer I to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 10 min. Remove the wash buffer. Repeat 2 times for a total of 3 washes.
22. Add enough 1X Wash Buffer II to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 5 minutes. Remove the wash buffer. Repeat one time for a total of two washes for 5 minutes per wash.
23. Finally, wash the glass slide with 30 ml of ddH₂O for 5 minutes. Remove glass slide and decant water from Centrifuge Tube.
24. Remove buffer droplets from the slide completely by one of the following ways:
- Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
 - Or dry the glass slide by a compressed N2 stream.
 - Or gently apply suction with a pipette to remove buffer droplets. Do not touch the array surface, only the sides.

Note: Make sure the finished glass slide is completely dry before scanning or storage.

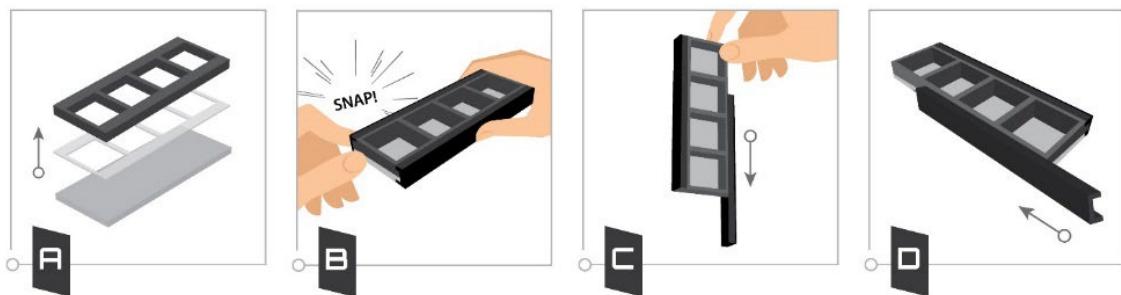
E. Fluorescence Detection

25. You may proceed immediately to scanning or you may store the slide at -20 °C in the Centrifuge Tube provided or at RT to scan at a later time.

Note: Please protect the finished glass slides from temperatures above RT and store them in the dark. Do not expose glass slide to strong light, such as sunlight or a UV lamp.

Note: If you need to repeat any of the incubation steps after finishing the experiment, you must first re-assemble the glass slide into the incubation chamber by following the steps as described below. To avoid breaking the printed glass slide, you may first want to practice assembling the device with a blank glass slide.

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



V. Antibody Array Map

VI. Antibody Array Target List

A. RayBio® Mouse Antibody Array L-308 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	6Ckine	63	DPPIV	125	IGFBP-1	187	IL-28B	249	SCF R
2	Activin A	64	DR3	126	IGFBP-2	188	IL-31	250	SDF-1
3	Activin C	65	Dtk	127	IGFBP-3	189	IL-31 RA	251	SAA1
4	Activin R1B	66	EDAR	128	IGFBP-5	190	Insulin	252	Shh-N
5	Adiponectin	67	EGFR	129	IGFBP-6	191	Integrin beta-2	253	SIGIRR
6	AgRP	68	EG-VEGF	130	IGFBP-L1	192	I-TAC	254	SLPI
7	ALCAM	69	Endocan	131	IGF-1	193	GRO alpha	255	Soggy-1
8	ANGPTL2	70	Endoglin	132	IGF-2	194	Kremen-1	256	SPARC
9	ANGPTL3	71	Endostatin	133	IL-1 alpha	195	Kremen-2	257	Spinesin
10	Amphiregulin	72	Eotaxin-1	134	IL-1 beta	196	Lefty-1	258	TACI
11	Artemin	73	Eotaxin-2	135	IL-1 R4	197	Leptin R	259	TARC
12	Axl	74	Epigen	136	IL-1 R6	198	LEPTIN	260	TCA-3
13	bFGF	75	Epiregulin	137	IL-1 R9	199	LIF	261	IL-27 R alpha
14	B7-1	76	Erythropoietin	138	IL-1 R1	200	LIGHT	262	TECK
15	BAFF R	77	E-Selectin	139	IL-1 R2	201	LIX	263	TFPI
16	BCMA	78	FADD	140	IL-2	202	LRP-6	264	TGF beta 1
17	beta-Catenin	79	FAM3B	141	IL-2 R alpha	203	L-Selectin	265	TGF beta 2
18	BLC	80	Fas	142	IL-2 R beta	204	Lungkine	266	TGF beta 3
19	Betacellulin	81	Fas Ligand	143	IL-3	205	Lymphotactin	267	TGF beta R1
20	Cardiotrophin-1	82	Fc gamma RIIB	144	IL-3 R alpha	206	LTBR	268	TGF beta R2
21	IL-1ra	83	FGF R3	145	IL-3 R beta	207	MAdCAM-1	269	TSP-1
22	CCL28	84	FGF R4	146	IL-4	208	MCP-1	270	CXCL7
23	MIP-1 beta	85	FGF R5 beta	147	IL-4 R	209	MCP-5	271	Tie-2
24	MCP-3	86	FGF-21	148	IL-5	210	M-CSF	272	TIMP-1
25	MCP-2	87	Flt-3 Ligand	149	IL-5 R alpha	211	MDC	273	TIMP-2
26	CCR10	88	FLRG	150	IL-6	212	MFG-E8	274	TIMP-4
27	CCR3	89	Follistatin-like 1	151	IL-6 R	213	MFRP	275	TL1A
28	CCR4	90	Fractalkine	152	IL-7	214	MIG	276	TLR1
29	CCR6	91	Frizzled-1	153	IL-7 R alpha	215	MIP-1 alpha	277	TLR2
30	CCR7	92	Frizzled-6	154	IL-9	216	MIP-1 gamma	278	TLR3
31	CCR9	93	Frizzled-7	155	IL-9 R	217	MIP-2	279	TLR4
32	CD11b	94	Galectin-3	156	IL-10	218	MIP-3 alpha	280	TMEFF1
33	CD14	95	GCSF	157	IL-10 R alpha	219	MIP-3 beta	281	TNF RI
34	CRP	96	GDF-1	158	IL-11	220	MMP-2	282	TNF RII
35	CD27	97	GDF-3	159	IL-12 p40	221	MMP-3	283	TNF alpha
36	CD27 Ligand	98	GDF-5	160	IL-12 p70	222	MMP-9	284	TNF beta
37	CD30	99	GDF-8	161	IL-12 R beta 1	223	MMP-12	285	Thrombopoietin
38	CD30 Ligand	100	GDF-9	162	IL-13	224	MMP-14	286	TRAIL
39	CD40	101	GFR alpha-2	163	IL-13 R alpha 2	225	MMP-24	287	TRAIL R2
40	CD40 Ligand	102	GFR alpha-3	164	IL-15	226	NRG3	288	TRANCE
41	Cerberus 1	103	GFR alpha-4	165	IL-15 R alpha	227	Neurturin	289	TREM-1
42	Chordin-Like 2	104	GITR	166	IL-16	228	NGFR	290	TROY
43	F3	105	GI/TR Ligand	167	IL-17A	229	NOV	291	TSLP
44	IL-2 R gamma	106	Glut2	168	IL-17 RB	230	Osteoactivin	292	TSLP R
45	IP-10	107	GM-CSF	169	IL-17C	231	Osteopontin	293	TWEAK
46	Cripto-1	108	Granzyme B	170	IL-17D	232	Osteoprotegerin	294	TWEAK R
47	Crossveinless-2	109	Granzyme D	171	IL-17E	233	OX40 Ligand	295	Ubiquitin+1
48	Cryptic	110	Granzyme G	172	IL-17F	234	PDGF-C	296	uPAR
49	CSK	111	Gremiin-1	173	IL-17 RA	235	PDGF R alpha	297	Urokinase
50	CTACK	112	GHR	174	IL-17 RC	236	PDGF R beta	298	VCAM-1
51	CTLA-4	113	HGFR	175	IL-17 RD	237	Pentraxin-3	299	VE-Cadherin
52	CXCL14	114	HGF	176	IL-18 R alpha	238	PF4	300	VEGF-A
53	CXCL16	115	HVEM	177	IL-20	239	PIGF-2	301	VEGFR1
54	CXCR2	116	ICAM-1	178	IL-20 R alpha	240	Progranulin	302	VEGFR2
55	CXCR3	117	ICAM-2	179	IL-21	241	Prolactin	303	VEGFR3
56	CXCR4	118	ICAM-5	180	IL-21 R	242	P-Selectin	304	VEGF-B
57	CXCR6	119	ICK	181	IL-22	243	RAGE	305	VEGF-C
58	EGF	120	IFN-alpha/beta R1	182	IL-22BP	244	RANTES	306	VEGF-D
59	Decorin	121	IFN-alpha/beta R2	183	IL-23	245	RELM beta	307	WIF-1
60	DKK-1	122	IFN-beta	184	IL-23 R	246	Resistin	308	WISP-1
61	Dkk-3	123	IFN-gamma	185	IL-24	247	S100A10		
62	Dkk-4	124	IFN-gamma R1	186	IL-27	248	SCF		

VII. Interpretation of Results:

A. Explanation of Controls Spots

There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized biotinylated IgG. 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. Some arrays may have beta-actin and GAPDH as internal controls, much as “housekeeping” genes or proteins are used to normalize results in PCR or Western blots, respectively.

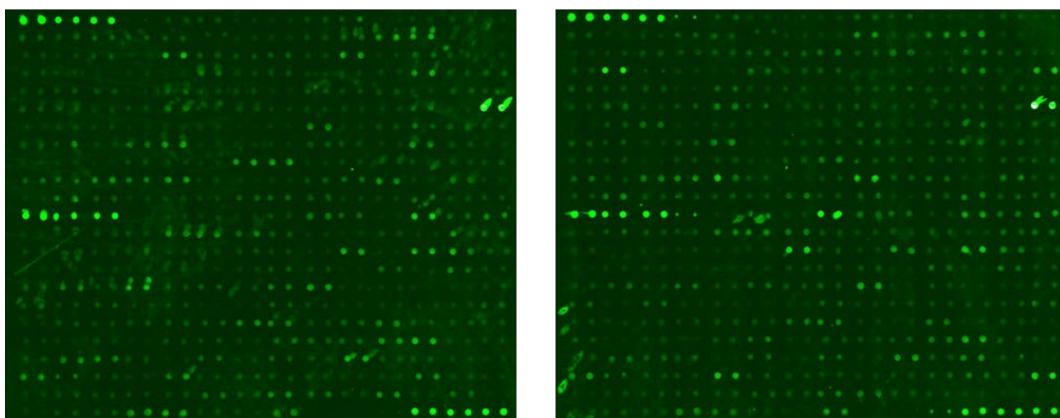
B. Typical Results

The following figure shows the typical result of this array probed with sample(s). The images were captured using an Axon GenePix laser scanner. The Positive control signals in the upper left and lower right corners of each array can be used to identify the orientation and help normalize the results between arrays.

RayBio® Mouse Antibody Array L-308

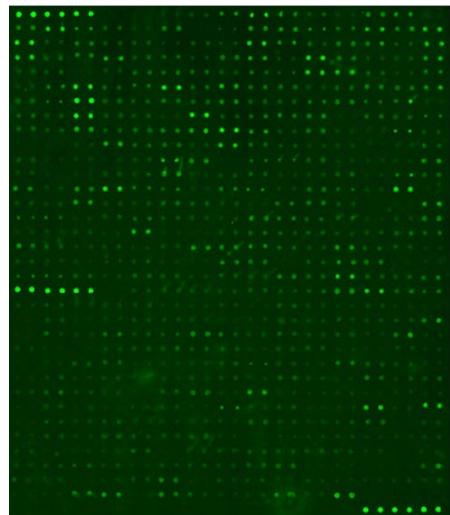
Serum

Plasma

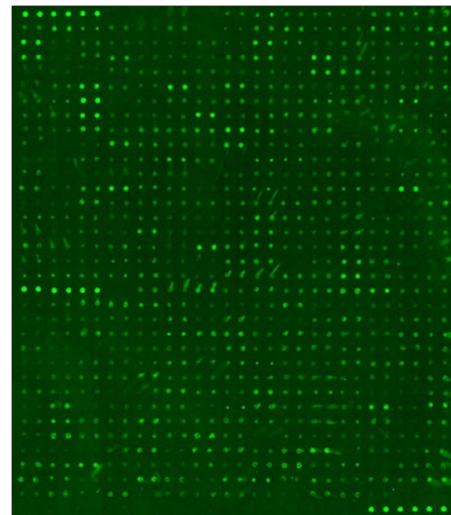


RayBio® Mouse Antibody Array L-2

Serum

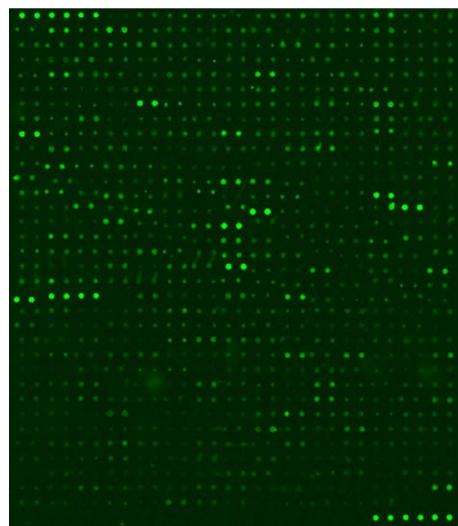


Plasma

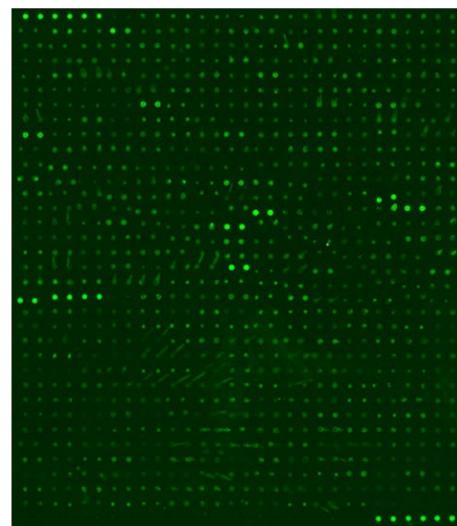


RayBio® Mouse Antibody Array L-3

Serum



Plasma



Note: 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 (i.e., concentrations of the various analytes in your samples), try using our Quantibody® Arrays as a targeted follow-up experiment.

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 scanners' software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEAN signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "F532 Mean - 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 freely 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. Analysis Tool software can be downloaded from the product page on the RayBiotech website.

E. Threshold of Significant Difference

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 $\approx 95\%$).

F. Pathway Analysis of the Array Proteins

Mouse antibody array L-2808 detects 2808 unique mouse proteins, including most analyzed cytokines, chemokines, adipokines, extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins. All the array proteins are provided with their Uniprot number and GeneID, which are essential for further data mining. Raybiotech offers affordable biostatistics and bioinformatics service, including data clean-up, differential expression analysis, cluster analysis, biomarker selection, pathway analysis and experimental design. See more details on the website: <https://www.raybiotech.com/biostatistics-and-bioinformatics-services>

VIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Dilute starting sample less or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
General	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from drying out during experiment

IX. Selected References

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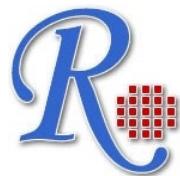
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