RayBio® Label-Based (L-Series) Mouse Antibody Array 308 (L-308)

Patent Pending Technology
User Manual (Revised Dec 9, 2019)

For the simultaneous detection of the relative expression of 308 (L-308) mouse proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Mouse Antibody Array L-308
Cat# AAM-BLG-1-4 (4 Sample Kit)
Cat# AAM-BLG-1-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

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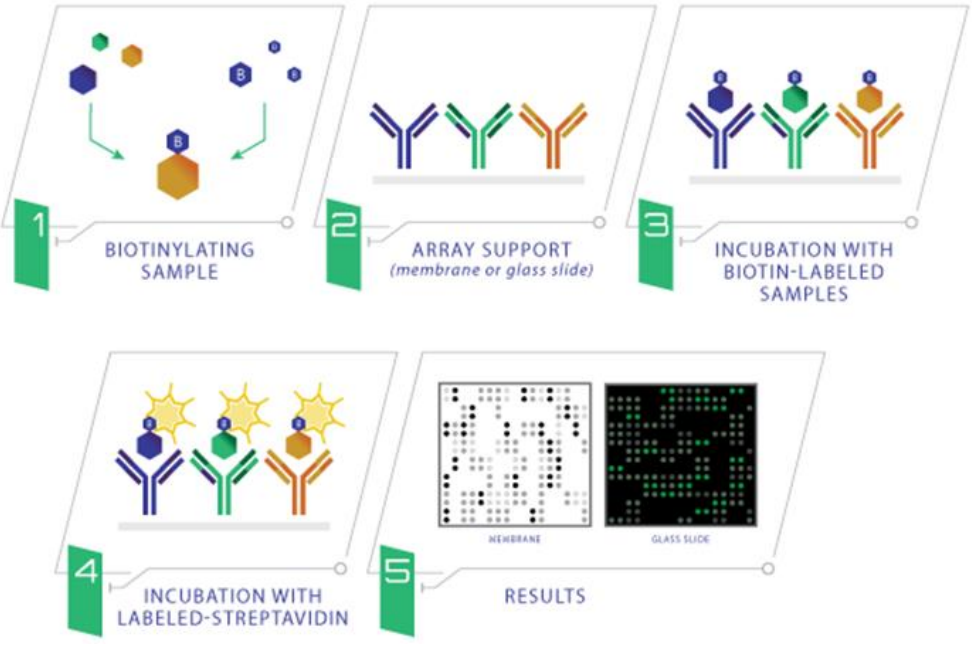
I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the L-Series Antibody Array 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 supernatants, serum and plasma.

The first step in using the RayBio® L-Series Mouse Antibody Array 308 is to biotinylate the primary amine of the proteins in serum or plasma samples, cell culture supernatant, cell lysate or tissue lysate. 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, and incubated to allow for interaction 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. 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 (may be stored for up to 3 months, Labeling Reagent, Item B should be fresh preparation before use). Unused glass slides should be kept at -20 °C and avoid repeated freeze-thaw cycles (may be stored for up to 6 months).

RayBio® L-Series Mouse Antibody Array 308

ITEM	DESCRIPTION	AAM-BLG-1-4	AAM-BLG-1-8
A	Dialysis Vials & Floating Dialysis Rack	8 vials	16 vials
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio® L-Series Mouse Antibody Array L-308 Glass Slides*	1 slide (L-308)	2 slides (L-308)
F	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml/ea.)
G	20X Wash Buffer I	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer II	1 bottle (30 ml)	1 bottle (30 ml)
I	Cy3-Conjugated Streptavidin	1 vial	2 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (8 ml)	
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	
M	30 ml Centrifuge Tube	1 tube	

*Each slide contains 4 identical subarrays
**Only needed if testing cell or tissue lysates

B. 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 available online)
- Aluminum foil

III. Overview and General Considerations

A. Preparation and Storage of Samples

1) Preparation of Cell Culture Supernatants

- Seed cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes (*).
- Culture in complete culture medium for ~24–48 hours (**).
- Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours (**, †). Recommended using membrane-based array if using high serum medium such as 10% FCS/FBS, the glass slide arrays tend to have extremely high background for high serum containing media samples.
- To collect supernatants, centrifuge at 1,000 g for 10 min and store as ≤1 ml aliquots at -80°C until needed.
- 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 vary and will depend on the cell line, treatment conditions and other factors.*

† *Bovine serum proteins produce detectable signals on the RayBio® L-Series Mouse Antibody Array 308 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

- For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS.
  For suspension cells, pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 min.
- Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.
- Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfugetubes and centrifuge at 13,000 rpm for 10 min at 2-8 °C *.
- Transfer supernatant to a clean tube. Determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at – 70°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 -70°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20

minutes at 2-8°C.

3) Extracting Protein from Crude Tissue

- 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 deionized or distilled water).
- Homogenize the tissue according to homogenizer manufacturer instructions.
- Transfer extracts to microcentrifuge tubes and centrifuge for 20 min at 13,000 rpm (4°C).
- Transfer supernatant to a clean tube and store at – 70°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 -70°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

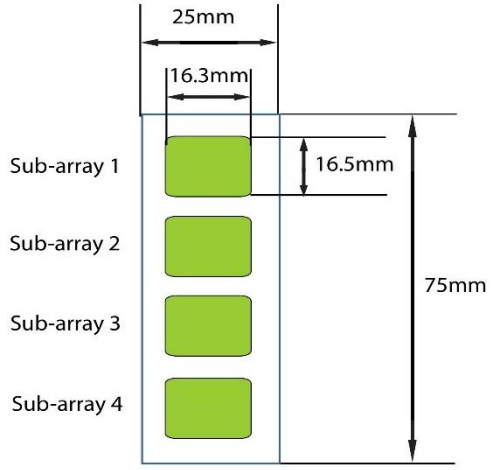
B. Handling the glass slides

- 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 19, and take great care not to break the glass slide when doing so.
- Permanent marker ink can significantly interfere with fluorescent signal detection. Never mark anywhere on the front (arrayed) side of the slide. It's best to avoid using marker completely, however if you need to number the slide, please add a small mark only on the back of the slide along the top or bottom edge using a green or blue ultra-fine point Sharpie® brand marker, only after the slide is completely dry.

- 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.

C. Layout of Mouse L-308 Glass Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

D. Incubations and Washes

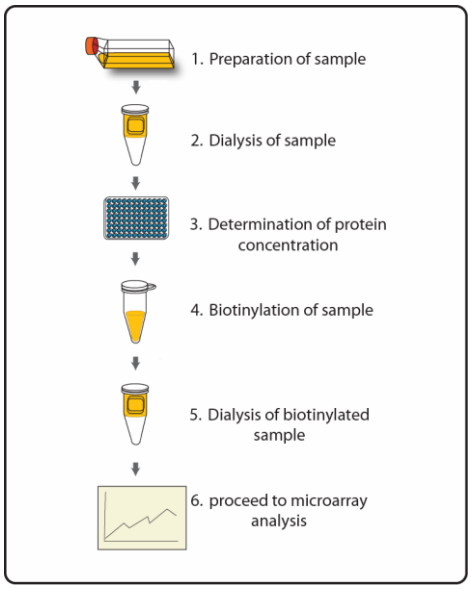
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and be sure to remove all bubbles from the sub-array surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/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 RT and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from directly strong light and temperatures above RT.

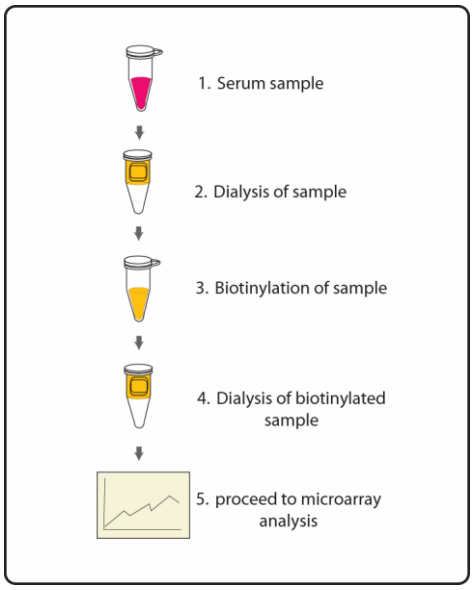
IV. Protocol

Assay Diagram

1. Cell culture supernatants or cell/tissue lysates*.



2. Serum or plasma



* If using cell or tissue lysates start at step 2. “Dialysis of sample”

A. 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 Dialysis Tube (Item A). Load 200 µl cell culture supernatant or 100 µl cell lysates or tissue lysate (1~2 mg/ml total protein) or 20 µl serum or plasma + 80 µl dialysis buffer (5-fold dilution. Carefully place Dialysis Tubes into Floating Dialysis Rack (Item L).

3. Place Floating Dialysis 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 dialysis 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 supernatants to a clean tube.

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

Note: Determine the total protein concentration for cell culture supernatants or cell/tissue lysate after dialysis procedure (Step 3). We recommended using a BCA total protein assay (eg, Pierce, Catalog # 23227).

B. 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 supernatants:** 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 supernatant. Mix well. For example, if sample's total protein concentration is 0.5 mg/ml you need to add 3.24 µl 1X Labeling Reagent to 180 µl dialyzed sample.

   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 Labeling 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/plasma and Labeling Buffer to compensate (to keep same total protein amount and total volume). For example, if serum/plasma sample volume increased from 100 µl to 200 µl, add 70 µl dialyzed serum and 120 µl Labeling Buffer to keep same total volume, 212 ul.*

   c) **For labeling cell or tissue lysates**: transfer 30 µg (15 µl of 2

mg/ml) cell or tissue lysates into a tube and add labeling buffer (Item K) for a total volume of 300 µl. Then add 3.3 µl of 1X Labeling Reagent Solution.

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. Make more dialysis buffer as directed in step 1. Collect each sample from reaction tube and add each sample into a separate Dialysis Tube (Item A). Immediately dialyze samples as directed in Step 3 on pages 8-9.

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

C. Drying of 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 approx. 15 min, and allow the Assembled Glass Slide to equilibrate to room temperature (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 <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 Assembled Glass Slide and incubating at RT for 30 min. Ensure there are no bubbles on the array surfaces.

11. Immediately prior to sample incubation, spin biotin-labeled samples for 5 min at 10,000 rpm to remove 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 supernatants, 20-fold for serum/plasma or 30-fold cell/tissue lysate .*

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 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: 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 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 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 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 min per wash.

16. 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 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) Add 200 µl of Cy3-Conjugated Streptavidin stock solution into a tube with 800 µl of Blocking Buffer. Mix gently to prepare 1X Cy3-Conjugated Streptavidin.

17. 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.

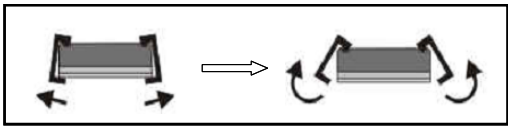
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in dark room.

18. Incubate with Cy3-Conjugated Streptavidin 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 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.



20. Gently place the glass slide into 30 ml Centrifuge Tube (Item M). Add enough 1X Wash Buffer I to cover the entire glass slide. 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 20, 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 slide with 30 ml of de-ionized or distilled water for 5 min. Remove glass slide and decant water from Centrifuge Tube.

23. Remove water droplets by applying suction gently with a pipette tip. Make sure the finished glass slide is completely dry before scanning or storage.

Note: Be careful not to touch the array portions of the slide with your pipette tip, only touch the sides of the slide. Alternatively, you may

gently dry the glass slide using a low-velocity Nitrogen gas stream or ambiently in a laminar flow hood or similar clean environment (Be sure to protect from light).

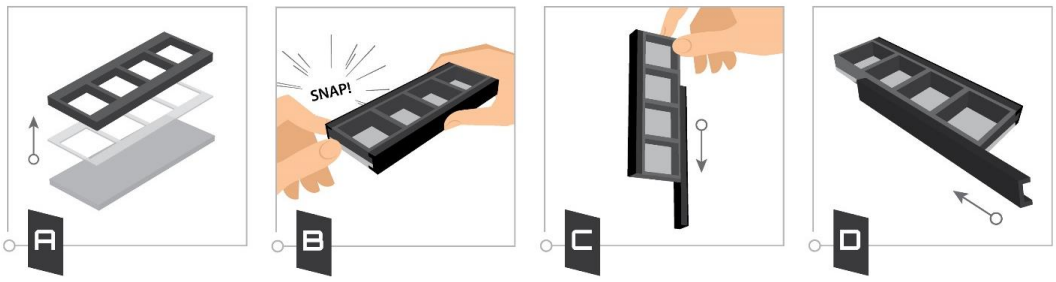
E. Fluorescence Detection

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 streptavidin-conjugated fluor used in this kit is very stable at RT and resistant to photobleaching on completed glass slides. However, please protect glass slides from temperatures above RT and store them in the dark. Do not expose glass slide 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 slide into the incubation chamber by following step as shown in the figures 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 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)*



V. Antibody Array Map

A. RayBio® L-series Mouse Antibody Array L-308 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
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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
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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® L-series Mouse Antibody Array L-308 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 / MT5-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	Osteoporotegerin	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	Epiregulin	135	IGF-I	191	IL-23	247	PlGF-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 rp2	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	FCrRIIB / 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	S100A10	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	Lymphotactin	269	TCCR / WSX-1	325	
46	Chordin-Like 2	102	GDF-5	158	neg	214	Lymphotoxin beta R / TNFRSF3	270	TECK	326	
47	Coagulation Factor III / Tissue Factor	103	GDF-8	159	IL-6 R	215	MAdCAM-1	271	TFPI	327	
48	Common 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	

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.

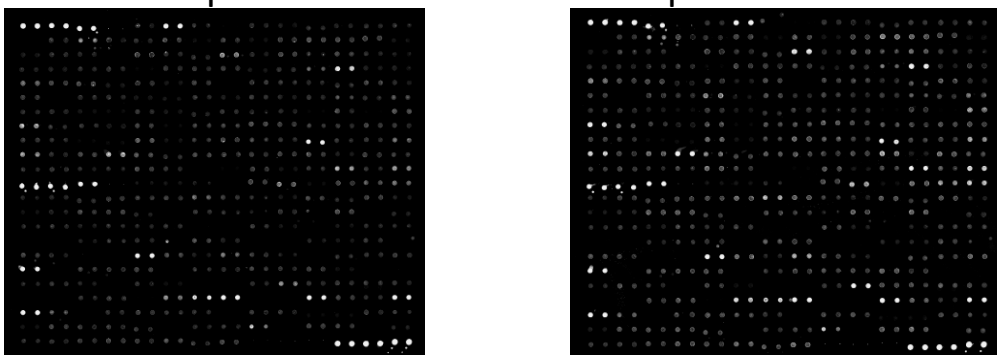
2) **Negative Control (NEG) spots** contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of Biotin-conjugated anti-Cytokines and/or the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical results obtained with RayBio® L-Series Mouse Antibody Array L-308

The following figure shows the RayBio® L-Series Mouse Antibody Array 308 probed with serum samples. The images were captured using an Axon GenePix laser scanner. The strong signals in row 20 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.

RayBio® L-Series Mouse Antibody Array 308

Sample-1 Sample-2



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 (i.e., 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%).

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	Avo id bubble formation during incubation
	Arrays are not completely covered by reagent	Completely cover arrays with solution

VIII. Selected References

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