RayBio® Label-Based (L-Series) Mouse Antibody Array L-3

Patent Pending Technology

User Manual (Revised Dec 9, 2019)

For the simultaneous detection of the relative expression of 500 mouse proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Mouse Antibody Array L-3
Cat# AAM-BLG-3-4 (4 Sample Kit)
Cat# AAM-BLG-3-8 (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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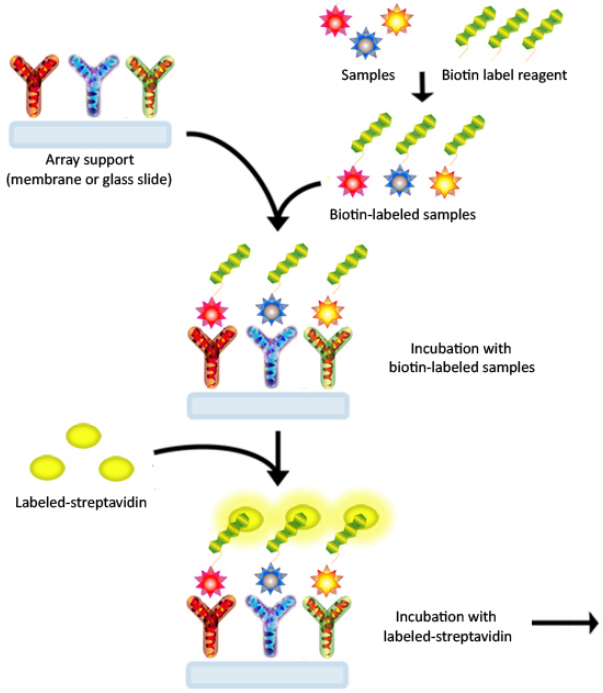
I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the L-Series Mouse Antibody Array L-3, researchers can now obtain a broad, panoramic view of protein expression. The expression levels of 500 target proteins can be simultaneously detected, including extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins in cell culture supernatants, cell lysate, tissue lysate, serum and plasma.

The first step in using the RayBio® L-Series Mouse Antibody Array L-3 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. Use within 6 months from the date of shipment is recommended. 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	AAM-BLG-3-4 (L-3)	AAH-BLM-3-8 (L-3)
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-3 Glass Slides*	1 slide (L-3)	2 slides (L-3)
F	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml)
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

- KCl, NaCl, KH_2PO_4, Na_2HPO_4 and ddH_2O
- 1 ml tube, small plastic or glass containers
- Orbital shaker or oscillating rocker
- Beaker, stir plate and stir bar
- 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

1. Seed cells at a density of $1x10^6$ 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 g for 10 minutes 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 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. Centrifuge 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. Proceed to b. Cells in Suspension.

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 ddH_2O). Solubilize the cells at $2x10^7$ 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. Determine 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 approximately 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O).

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.

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.

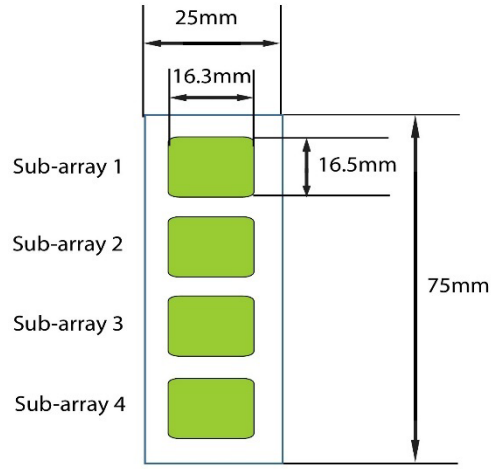
- Handle the slides with powder-free gloves and in a clean environment.

- Do not remove the glass slide from the chamber assembly until step 20 on page 15, 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 as seen in image below.

C. Layout of Mouse L-3 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 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.

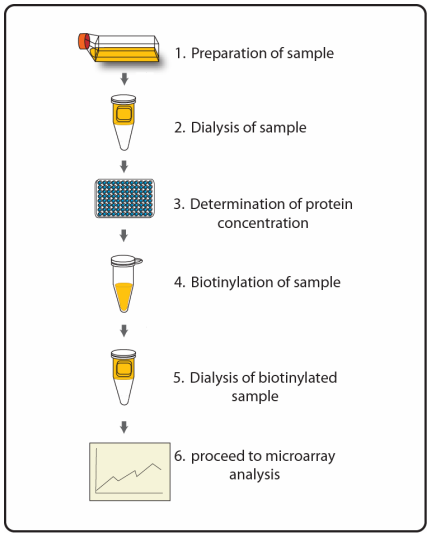
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.

- 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 directly strong light and temperatures above RT.

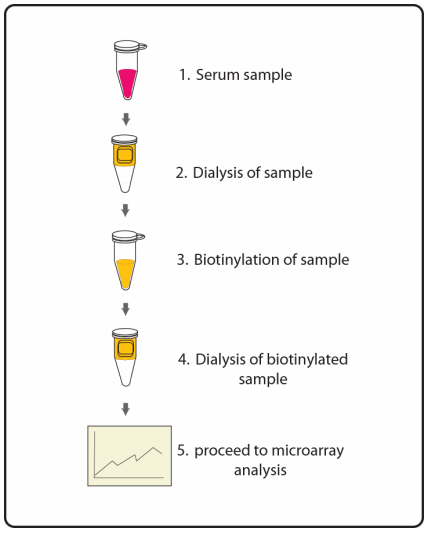
IV. Protocol

Assay Diagram

1. Cell culture supernatants or cell/tissue lysates



2. Serum or plasma



Note: If using cell or tissue lysates, start at "Dialysis of sample"

A. Dialysis of Sample

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

1. Prepare enough dialysis buffer (1X PBS, pH=8.0) for all dialysis steps herein and after. To prepare 1 L dialysis buffer, dissolve 0.2 g KCl, 8 g NaCl, 0.2 g KH_2PO_4 and 1.15 g Na_2HPO_4 in 800 ml ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 ml with ddH_2O.
2. Add each sample into a separate Dialysis Tube (Item A). Loading volumes are as follows: 200 µl cell culture supernatant; 100 µl cell or tissue lysate (1-2 mg/ml total protein); 20 µl serum or plasma + 80 µl dialysis buffer (5-fold dilution). Carefully place Dialysis Tubes into Floating Dialysis Rack.

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

3. Place Floating Dialysis Rack into ≥500 ml dialysis buffer in a large beaker. For more than 2 samples, make certain to use at least 300 ml dialysis buffer for each sample (more buffer will improve the efficiency of dialysis). 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 hours at 4˚C. Transfer dialyzed sample to a clean microfuge tube. Spin dialyzed samples for 5 min at 10,000 rpm to remove any particulates or precipitates, 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 (e.g., Pierce, Catalog # 23227).

B. Biotin-labeling of 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, then 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 the tube of 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).

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 260 µl. Then add 3.3 µl of 1X Labeling Reagent Solution.

Note: To normalize serum/plasma or cell/tissue lysate concentrations during biotinylation, measure sample volume before and after dialysis. Then adjust the volumes of dialyzed serum/plasma or cell/tissue lysates and Labeling Buffer to compensate. For example, if the sample volume doubles after dialysis, then use twice as much serum/plasma in the labeling reaction (70 µl) and reduce the Labeling Buffer to 120 µl.

6. Incubate the reaction solution at RT with gentle rocking or shaking for 30 minutes. Mix the reaction solution by gently tapping the tube every 5 minutes.

7. Add 3 µl Stop Solution (Item D) into each reaction tube. Collect and transfer each sample from reaction tube into a separate Dialysis Tube (Item A). Immediately dialyze samples as directed in Step 3 on pages 9.

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 min. Ensure there are no bubbles on the array surfaces.

11. Immediately prior to sample incubation, spin biotin-labeled samples for 5 minutes at 10,000 rpm to remove any particulates or precipitates. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 2-10-fold for cell culture supernatants, 20-fold for serum/plasma and 30-fold for 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. 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 ddH_2O.

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 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.

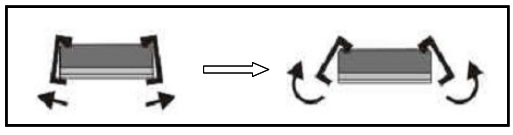
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 2 hours 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 minutes. Remove the wash buffer. Repeat 2 times for a total of 3 washes.

22. Repeat step 20, this time with 1X Wash Buffer II. Repeat one time for a total of two washes for 5 minutes per wash.

23. Finally, wash the glass slide with 30 ml of ddH_2O for 5 min. 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 slides in a laminar flow hood for 20 minutes or until slide is completely dry.
    - 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, only the sides.

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

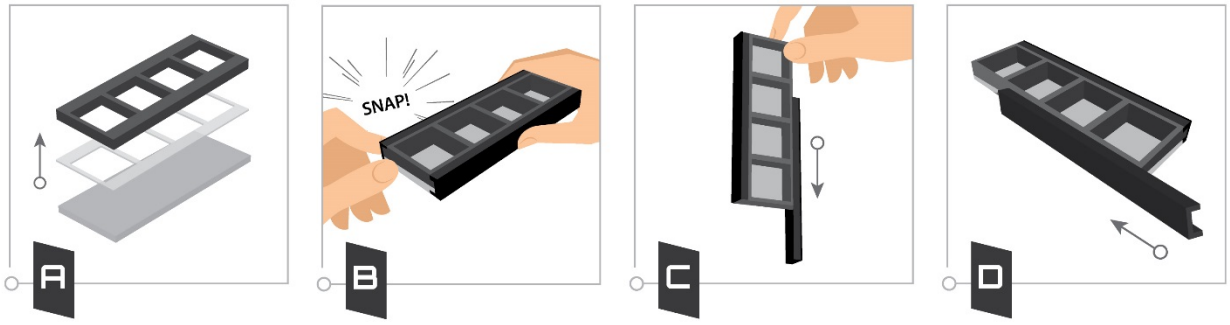
E. Fluorescence Detection

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: <u>Please protect the finished glass slides from temperatures above RT and store them in the dark</u>. 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 and Target List

A. RayBio® Mouse Antibody Array L-3 Map

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B. RayBio® Mouse Antibody Array L-3 Target List

number	name	number	name	number	name	number	name	number	name
1	Pos 1a	61	Dematin	121	Myosin IIB	181	PFAS	241	PSMB1
2	Pos 2a	62	DIAPH1	122	NACA1	182	PFDN6	242	PSMB2
3	Pos 3a	63	DKC1	123	NAGPA	183	PFKL	243	PSMB3
4	Neg	64	DLST	124	NAV2	184	PGAM1	244	PSMB4
5	AARE	65	DMRT1	125	NFATC4	185	PGAM2	245	PSMB5
6	ACAT1	66	Dystrophin	126	NNT	186	PGK-1	246	PSMB6
7	acyl-CoA Thioesterase 2	67	Ebf4	127	NPEPPS	187	PGLS	247	PSMB7
8	ADAM28	68	EBP50	128	NQO2	188	PG-M	248	PSMC3
9	AHCY	69	ECHDC1	129	NSFL1C	189	PGM1	249	PSMD1
10	AK1	70	EHHADH	130	Nucleobindin 1	190	PGRPL	250	PSMD5
11	AKR1A1	71	EIF3D	131	NUP214	191	PHGDH	251	PSMD9
12	ALDH2	72	eIF4A2	132	OAF	192	Piccolo	252	PSME1
13	alpha 5 D	73	eIF4GII	133	OIT3	193	pIgR	253	PSME2
14	ANKRD9	74	ENDOD1	134	OPCML	194	PIK3C2B	254	PTBP1
15	Annexin A3	75	EYA2	135	Orosomucoid 2	195	PIN	255	PTEN
16	AP180	76	Factor VIII	136	OSBP1	196	PIP5K2 alpha	256	PTGR1
17	AP3S2	77	Filaggrin	137	OSCAR	197	PISD	257	PTK 7
18	APLP2	78	FITM1	138	OSM R beta	198	PLA2G1B	258	PTMA
19	Apolipoprotein A V	79	GARS	139	Osteoadherin	199	Plastin 3	259	PTPRG
20	ASPM	80	GCC2	140	OTC	200	Plastin L	260	PTPRK
21	ASS1	81	GLI-2	141	OTUB1	201	PLBD2	261	PTPRM
22	ATOX1	82	GLOD4	142	OTUD7A	202	PLD4	262	PTPRZ
23	ATPG	83	GLUL	143	Oxytocin-neurophysin 1	203	Plectin	263	PZP
24	AUTS2	84	GMPR1	144	p16 ARC	204	Plexin B1	264	QARS
25	BAI2	85	GOLGA3	145	p23	205	Plexin B2	265	QDPR
26	BarX1	86	GP2	146	p39	206	PLOD1	266	QPRT
27	BBS1	87	gp340	147	P4HB	207	PLOD2	267	Quiescin Q6
28	BE2I / UBC9	88	GTF2F1	148	p73	208	Plxdc2	268	Rab1A
29	BLM	89	HA1	149	PABP1	209	PMCA	269	Rab7a
30	BOLA2	90	HARS	150	PACS1	210	PNP	270	Ran
31	C10orf58	91	HIC1	151	Pancreatic Lipase	211	POLD2	271	RanBP1
32	CACNA1H	92	HIP55	152	PARVB	212	POLR2A	272	RanGAP1
33	Calpain 2	93	Histone H1.0	153	PCAP	213	POR	273	RAP1B
34	CaMK2	94	Histone H1.5	154	PCBP1	214	PPOX	274	Rbm15
35	CaMK2D	95	HIVEP2	155	PCBP2	215	PPP1CC	275	RCL
36	CBL	96	hnRNP K	156	PCCA	216	PPP1R9A	276	RECQ4
37	CBR1	97	hnRNP R	157	PCDH12	217	PPP2R1B	277	Reg3A
38	CCDC58	98	HNRNPUL2	158	PCDH8	218	PPP2R4	278	REV3L
39	CCT6A	99	HNRPA3	159	PCK2	219	PRCP	279	RHOC
40	CHCHD3	100	HP1 g	160	PCMT1	220	PRDM13	280	RHOG
41	Cingulin	101	Importin 7	161	PCNA	221	PREP	281	Ribonuclease A
42	CIT	102	Involucrin	162	PCPE-1	222	PRG2	282	Ribonuclease T2
43	CMG1	103	ISLR	163	PCSK9	223	Prion protein PrP	283	RLF
44	CNBP	104	ITPR2	164	PDAP1	224	Profilin 1	284	RNASE4
45	CNPY2	105	ITPR3	165	PDE1B	225	Prolargin	285	Rnose2
46	Coilin	106	KCNAB3	166	PDIA6	226	Prosaposin	286	Pos 1b
47	COL8A2	107	-Laminin alpha 5	167	PDLIM1	227	Prostaglandin D Synthase	287	Pos 2b
48	COLEC11	108	LDB3	168	PDLIM3	228	Proteasome 26S S2	288	Pos 3b
49	COPG2	109	LHPP	169	PDZD2	229	Protein C	289	Neg
50	CORO1B	110	LIPG	170	PEBP1	230	Protein Z	290	RP1
51	CPA3	111	MAP4K4	171	PEBP4	231	PRR4	291	RPL10
52	CPI17 alpha	112	MICALL2	172	PENK	232	PRRC2A	292	RPL10A
53	CrkRS	113	MON2	173	PEPD	233	PRSS23	293	RPL11
54	CRLF3	114	MPST	174	perilipin 3	234	PRSS3	294	RPL12
55	CSRP3	115	MRC2	175	Perilipin-1	235	PRTN3	295	RPL14
56	CTNNAL1	116	MSH3	176	Periostin	236	PSMA1	296	RPL17
57	CTNND1	117	MTA2	177	Periplakin	237	PSMA2	297	RPL22
58	Cyclophilin F	118	MTHFD1	178	Peroxiredoxin 2	238	PSMA4	298	RPL23A
59	Cytochrome b5	119	MUC5B	179	Peroxiredoxin 3	239	PSMA5	299	RPL3
60	DCAMKL1	120	MVD	180	Peroxiredoxin-1	240	PSMA6	300	RPL32

RayBio® Mouse Antibody Array L-3 Target List…Continued

number	name	number	name	number	name	number	name
301	RPL4	361	SEZ6L2	421	TCTP	481	Uteroglobin
302	RPL7	362	SF20	422	TDIF2	482	Utrophin
303	RPL7A	363	SHANK1	423	Tenascin C	483	valyl tRNA
304	RPLP0	364	SHC1	424	Tenascin XB	484	VAP-1
305	RPLP2	365	SHMT1	425	TFF2	485	VAP-A
306	RPS10	366	SHOX	426	TGM3	486	VCP
307	RPS11	367	SHP-1	427	Thioredoxin-1	487	VDAC1
308	RPS12	368	Siglec-1	428	THOP1	488	VILIP3
309	RPS13	369	SIM2	429	TIF1 alpha	489	Vimentin
310	RPS14	370	SIRPB1	430	TMEM103	490	VNN1
311	RPS15A	371	Six3	431	TOB2	491	VPS4B
312	RPS16	372	SLC4A1	432	TOMM70A	492	VSIG4
313	RPS18	373	SLITRK1	433	TOP2B	493	WDR1
314	RPS19	374	SLURP1	434	TPD52L2	494	WDR44
315	RPS2	375	SMAD6	435	TPM4	495	WISP2
316	RPS20	376	SMC4	436	TPP1	496	WNK2
317	RPS23	377	SMPD4	437	TPPP3	497	XPG
318	RPS25	378	SNRPD1	438	TPR	498	YB1
319	RPS3	379	SOD1	439	Transaldolase 1/TALDO1	499	YN1 / Synapsin 1
320	RPS3A	380	SOD2	440	Transthyretin	500	YY1
321	RPS4X	381	SOD-3	441	TRAP1	501	ZAK
322	RPS5	382	Somatoliberin	442	TRAP220	502	zbtb11
323	RPS8	383	Somatostatin	443	TRF 2	503	ZBTB4
324	RPS9	384	SORD	444	TRIM14	504	ZC3H18
325	RREB1	385	SorLA	445	Tropomyosin 3	505	ZC3H4-N-t
326	RSF1	386	SOX4	446	TRP-1	506	ZC3H8
327	RSU1	387	SOX5	447	TRPS1	507	ZNF295
328	RUSC1	388	SP-D	448	Trypsinogeb-2	508	Zyxin
329	S E P T 7	389	Spectrin	449	TSR2	509	Neg
330	S100A1	390	SPEN	450	TTC3	510	Neg
331	S100A11	391	SPG48	451	TTF1	511	Neg
332	S100A7	392	SPINK5	452	TUBA6	512	Neg
333	S100A9	393	SPS2L	453	TWF2	513	Neg
334	SAA	394	SPTBN2	454	TXNDC15	514	Neg
335	SAA4	395	SPTLC1	455	TXNDC4	515	Neg
336	SBP-1	396	Src	456	TXNDC5	516	Neg
337	SC35	397	SSC5D	457	TXNRD2	517	Neg
338	SCG	398	STAT3	458	UBA1	518	Neg
339	SCN3A	399	Stathmin 1	459	UBE2D3	519	Neg
340	SCP2	400	STI1	460	Ube2L3	520	Neg
341	SDNSF	401	STOM	461	UBE2N/Ubc13	521	Neg
342	SDPR	402	STXBP2	462	UCH-L1	522	Neg
343	SECISBP2	403	SUCLG1	463	UFM 1	523	Pos 1c
344	Secretogranin V	404	SUMO3	464	UGGT	524	Pos 2c
345	Semaphorin 6B	405	SVEP1	465	UMP/CMP	525	Pos 3c
346	Semaphorin 7A	406	Symplekin	466	UNC13 Homolog D	526	
347	SERBP1	407	SynCAM	467	UNC45A	527	
348	Serpin A11	408	Synemin	468	UNC5H4	528	
349	Serpin A7	409	SYNPO2L	469	UPB1	529	
350	Serpin B3D	410	Syntaxin 7	470	UQCRB	530	
351	Serpin B6	411	TAB182	471	UQCRH	531	
352	Serpin B8	412	Talin1	472	URB	532	
353	Serpin F2	413	TARS	473	URB2	533	
354	Serpin H1	414	TAX1BP3	474	UROC1	534	
355	Serpin A10	415	TBCA	475	UROD	535	
356	SERPINB1	416	TCEB2	476	Uroguanylin	536	
357	SerpinB4	417	Tcf20	477	URP2	537	
358	SerpinE2	418	TCP1 delta	478	USP14	538	
359	SerRS	419	TCP1 eta	479	USP2	539	
360	SET	420	TCP1 theta	480	USP5	540	

VI. Interpretation of Results:

A. Explanation of Controls Spots

1) Positive Control spots (POS1, POS2, POS3) are standardized amounts of biotinylated IgG 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.

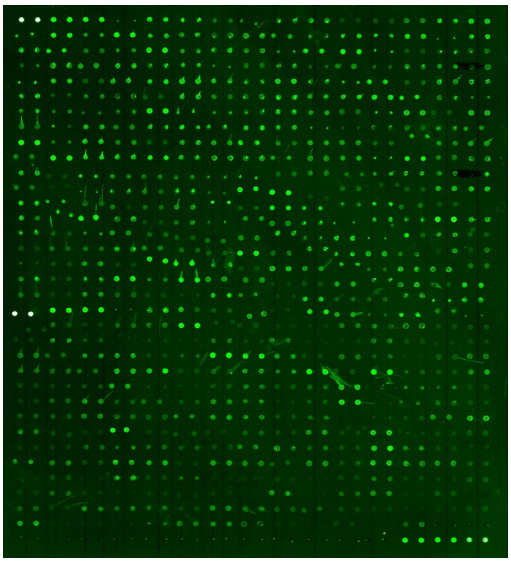
2) Negative Control (NEG) spots contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical Results

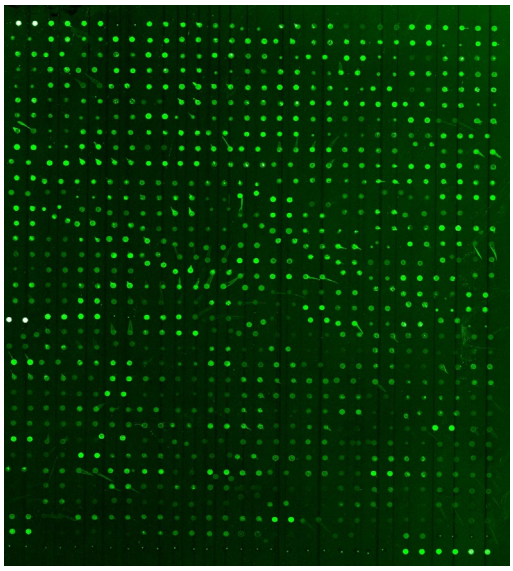
The following figure shows the RayBio® L-Series Mouse Antibody Array 3 probed with serum sample. The images were captured using an Axon GenePix laser scanner. The strong signals in row 20 and the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays.

RayBio® L-Series Mouse Antibody Array L-3

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.

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 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

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	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 completed 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 dying out during experiment

VIII. Selected References

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