

RayBio[®] Label-Based (L-Series) Rat L3 Array, Membrane

**Patent Pending Technology
User Manual (Jan 1, 2022)**

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

**AAR-BLM-3-2 (2 Sample Kit)
AAR-BLM-3-4 (4 Sample Kit)**

Please read manual carefully before starting experiment



Your Provider of Excellent Protein Array Systems and Services

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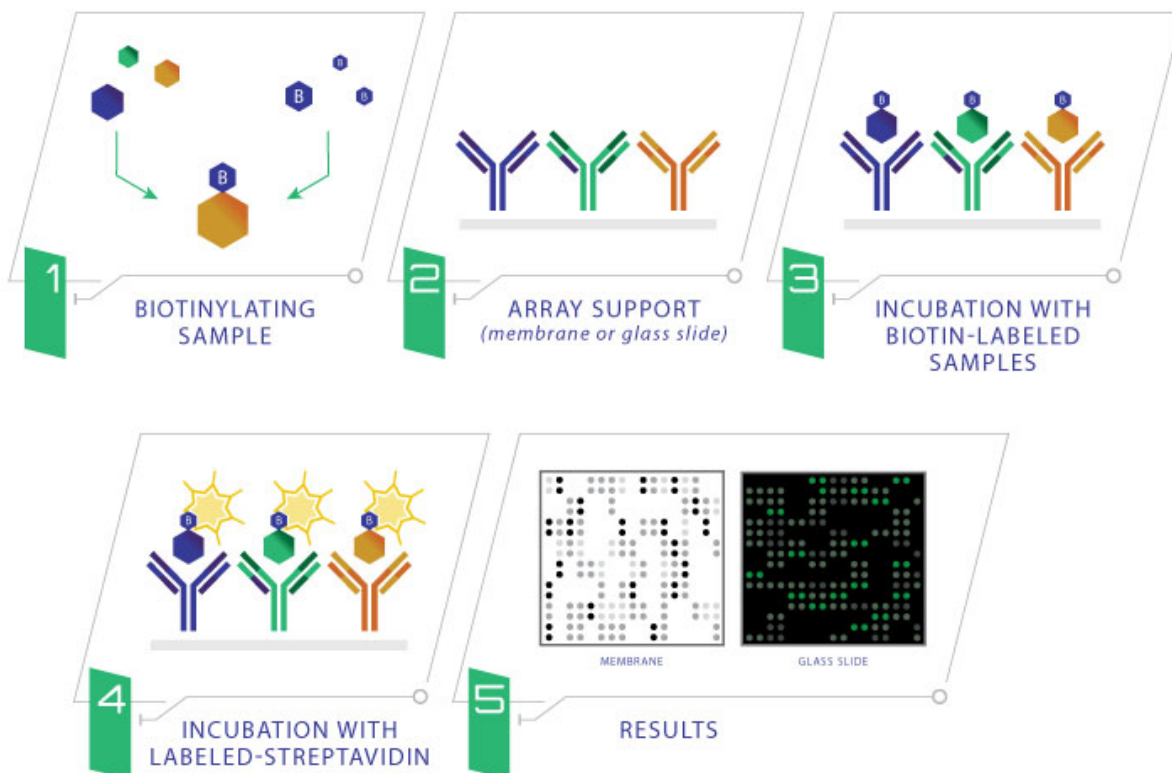
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I. Introduction

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 membrane arrays are then blocked, similar as a Western blot, and the biotin-labeled sample is added onto the membrane array which is pre-printed with capture antibodies and incubated to allow for interaction of target proteins. After incubation with HRP-Conjugated Streptavidin, the signals can be visualized by chemiluminescence.



II. Materials Provided

A. Storage Recommendations

Upon receipt, Box 1 should be stored at -20°C and Box 2 should be stored at 4°C . The kit must be used within 6 months from the date of shipment. After initial use, Blocking Buffer, Stop Solution, HRP-Conjugated Streptavidin, Detection Buffers C and D 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). The Array Membrane should be kept at -20°C and avoid repeated freeze-thaw cycles (may be stored for up to 6 months).

Box 1 (store at -20°C):

ITEM	DESCRIPTION	2 MEMBRANE KIT	4 MEMBRANE KIT
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 μl)	1 vial (50 μl)
E	L-series Antibody Array Membranes	2 membranes	4 membranes
F	4X Blocking Buffer	1 bottle (30 ml)	1 bottle (30 ml)
I	500X HRP-Conjugated Streptavidin Concentrate	1 vial (100 μl)	1 vial (100 μl)
K	Detection Buffer C	1 bottle (10 ml)	2 bottles (10 ml)
L	Detection Buffer D	1 bottle (10 ml)	2 bottles (10 ml)
Other Kit Components: Plastic Sheets			

Box 2 (store at 4°C):

ITEM	DESCRIPTION	2 MEMBRANE KIT	4 MEMBRANE KIT
G	20X Wash Buffer 1 Concentrate	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer 2 Concentrate	1 bottle (30 ml)	1 bottle (30 ml)
	Labeling Buffer	1 bottle (30 ml)	2 bottles (30 ml/ea)
J-2	Spin Columns	4 columns	8 columns
N/A	Plastic Incubation Trays (w/lid)	2 trays	4 trays
N/A	2X Lysis Buffer	1 bottle (10 ml)	1 bottle (10 ml)

B. Additional Materials Required

- 2-5 ml tube, small plastic or glass containers
- 15 ml conical collection tubes
- Orbital shaker or oscillating rocker
- Kodak X-OmatTM AR film (REF 165 1454) and film processor or Chemiluminescence imaging system
- Pipettors, pipette tips and other common lab consumables
- Eppendorf tube

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.**,+
4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as less than or equal 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 densitometry 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 2×10^7 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. 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).

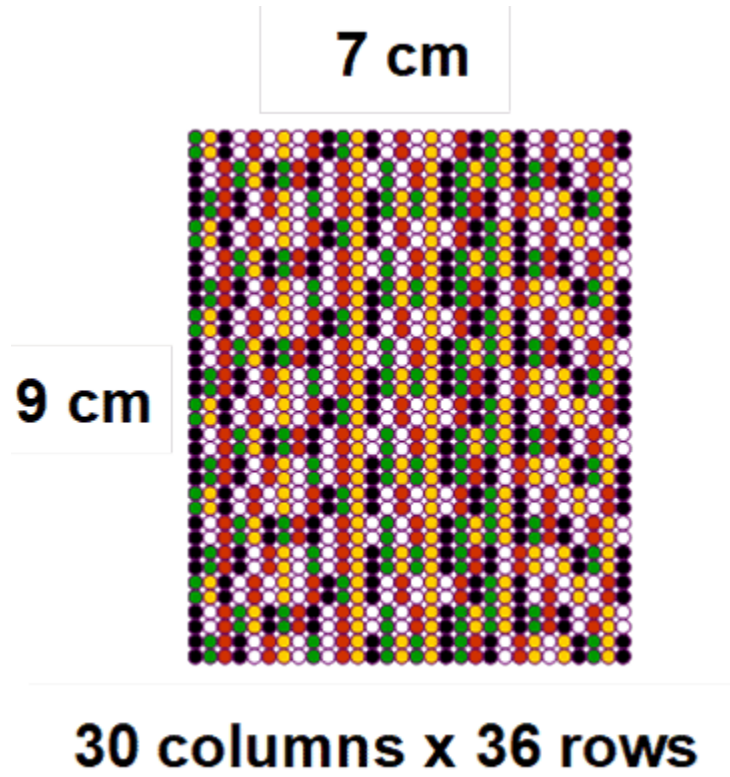
B. Handling the Array Membranes

- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

C. Incubations of Antibody Array

- Completely cover membranes with sample or buffer during incubation and cover the Plastic Incubation Tray with the lid to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Several incubation steps such as step 3 (sample incubation) or step 7 (HRP-Conjugated Streptavidin incubation) may be done at 4 °C overnight.

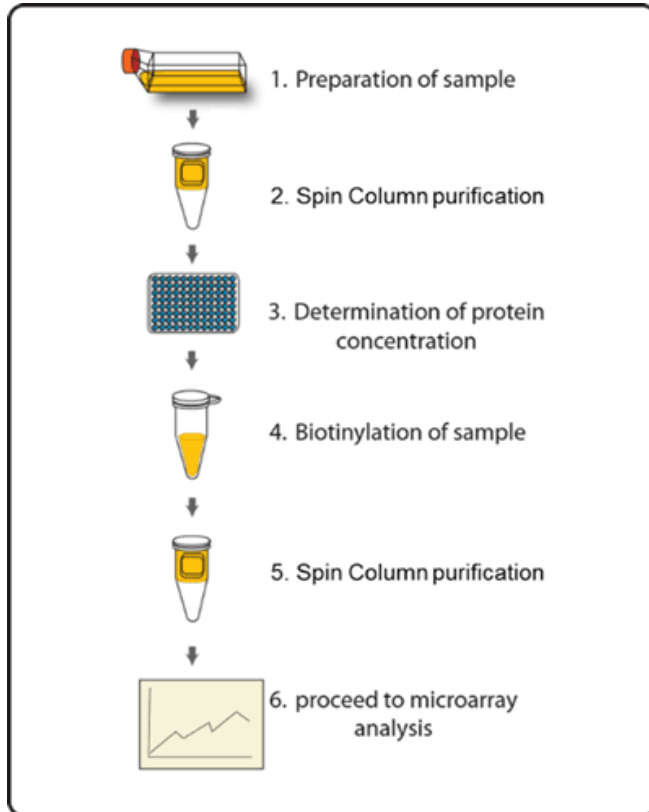
D. Layout of Array Membrane



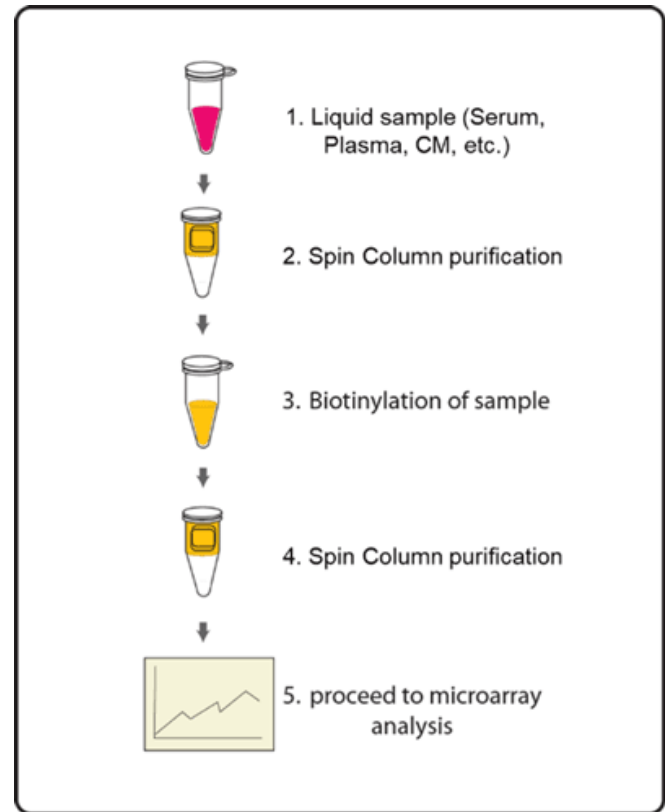
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



2. Serum, plasma, 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 15 ml conical collection tube, centrifuge at 1,000 x g for 3 minutes to remove the storage buffer. Discard the flow-through.
3. Wash the column three times with 1 ml labeling buffer each, centrifuge 1,000 x g for 3 minutes to remove the flow-through. Blot the bottom of the column to remove excess liquid, and transfer device to a new collection tube.
4. Apply sample on top of the resin within the next few minutes. Centrifuge at

1,000 x g for 3 minutes to collect the flow-through that contains sample. The recommended sample dilution as following:

- *Cell culture supernatant: 600 μ l neat supernatant*
- *Serum/Plasma: 10 μ l serum/plasma in 600 μ l Labeling Buffer*
- *Cell/tissue lysate: 100 μ g lysate in 500 μ l Labeling Buffer*

Note: The maximal sample volume is 700 μ l for each Spin Column. Do not load over 700 μ l 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 μ l 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 10 μ l of Labeling Reagent into the sample tube (for 600 μ l supernatant).
 - b. For labeling serum or plasma: Add 10 μ l of Labeling Reagent into the sample tube (for 10 μ l serum/plasma in 600 μ l labeling buffer).
 - c. For labeling cell or tissue lysates: Add 5 μ l of 1X Labeling Reagent into the sample tube (for 100 μ g lysate in 500 μ l labeling buffer).
 - d. For all other body fluid: Add 2 μ l of Labeling Reagent Solution per 100 μ g sample to be labelled.

Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If more or less amount sample is labelled, adjust this volume proportionally.

7. Add 5 μ l 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. Blocking and Incubations

8. Place each membrane printed side up into a Plastic Incubation Tray (provided). 1 membrane per tray.

Note: The printed membrane will have a "-" mark in the upper left corner of the membrane.

Note: Up to 4 membranes can be incubated together within one tray with proportional amount of reaction buffer. Rotate the membrane sequence at least once during sample incubation if more than one membrane is incubated in one tray.

9. Dilute 4X Blocking Buffer (Item F) with deionized or distilled water to prepare the 1X Blocking Buffer. Add 6 ml of 1X Blocking Buffer to each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour.
10. Aspirate the Blocking Buffer from each tray. Add 6 ml of diluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

Note: It is recommended to use 10-20 folds diluted biotin-labeled culture supernatant, 10-20 folds diluted biotin-labeled serum/plasma, 100 folds diluted biotin-labeled cell/tissue lysate, or 10-20 folds for other body fluid. Dilute sample using 1X Blocking Buffer. The optimal concentration of sample used will depend on the abundance of target proteins. The samples can be concentrated if the overall signals are too weak. If the overall signals are too strong, the sample can be diluted further.

Note: Incubation may be done at room temperature with gentle shaking for 2 hours or overnight at 4 °C.

11. Dilute 20X Wash Buffer 1 (Item G) with deionized or distilled water to prepare the 1X Wash Buffer 1. Aspirate the samples from each tray and then wash by adding 20 ml of 1X Wash Buffer I at room temperature with gentle shaking (5 min per wash). Repeat the wash 2 more times for a total of 3 washes.

12. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 (Item H) with deionized or distilled water to prepare the 1X Wash Buffer 2. Wash 3 times with 20 ml of 1X Wash Buffer 2 at room temperature with gentle shaking.
13. Aspirate the 1X Wash Buffer 2 from each tray.
14. Prepare the HRP-Conjugated Streptavidin. Briefly spin down the tube containing the 500X HRP-Conjugated Streptavidin (Item I) immediately before use. Dilute the 500X HRP-Conjugated Streptavidin with 1X Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. Pipette up and down to mix gently. Add 6 ml of 1X HRP-Conjugated Streptavidin to each membrane.

Note: Ensure that the vial containing the 500X HRP-Conjugated Streptavidin is mixed well before use, as precipitation can form during storage.

15. Incubate at room temperature with gentle shaking for 2 hours.

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

16. Wash as directed in steps 11 through 13.

D. Detection

Note: Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.

17. For detection of 2 membranes, add 4.2 ml of Detection Buffer C and 4.2 ml of Detection buffer D into a tube and mix both solutions. Drain off excess wash buffer. Place membrane antibody side up (There is a "-" symbol on the top left corner of each membrane) on a clean plastic plate or its cover (provided in the kit). Pipette 4 ml of the mixed Detection Buffers onto each membrane and incubate at room temperature for 2 minutes with gentle shaking. Ensure that the detection mixture is evenly covering the membrane without any air bubbles.
18. Gently place the membrane with forceps (antibody side up) on a plastic sheet (provided) and cover the membrane with another plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane. Work as quickly as possible.
19. The signal can be detected directly from the membrane using a chemiluminescence imaging system or by exposing the array to x-ray film (we recommend using Kodak X-Omat™ AR film) with subsequent development.

Expose the membranes for 40 seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce the exposure time (e.g., 5-30 seconds). If the signals are too weak, increase the exposure time (e.g., 5-20 min or overnight) or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

20. Save membranes at -20 °C to -80 °C for future reference.

V. Antibody Array Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
1	POS1	POS2	POS3	Blank	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				
2	POS1	POS2	POS3	Blank	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				
3	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53				
4	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53				
5	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83				
6	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83				
7	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113				
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35	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS3	POS2	POS1	
36	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS3	POS2	POS1

VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	A2M	73	CHGB	145	FGFR5	217	IL-1 R4	289	Neurturin	361	Quiescin Q6	433	TCP1
2	AARE	74	Chk1	146	FGG	218	IL-1 R6	290	Nidogen-1	362	RAGE	434	TDIF2
3	ABCF1	75	Chymase	147	FH	219	IL-11	291	Nidogen-2	363	Ras	435	TECK
4	ACAT1	76	CINC-2	148	Fibronectin	220	IL-12 p40	292	NIT2	364	RELM beta	436	Tenascin X
5	Activin A	77	CINC-3	149	Ficolin-2	221	IL-12 RB1	293	NNT	365	Resistin	437	TFF1
6	ADAMTS10	78	Cingulin	150	FLG2	222	IL-13	294	NOV	366	REV3L	438	TFF2
7	ADAMTS15	79	CIP29	151	FOXP3	223	IL-15	295	NPB	367	Rheb	439	TGF-beta 1
8	ADAMTSL2	80	Claudin-3	152	Fractalkine	224	IL-16	296	NPTXR	368	RNASE6	440	TGF-beta 2
9	Aggrecan	81	Claudin-4	153	Frizzled-1	225	IL-17A	297	NR3C3	369	ROBO4	441	TGF-beta 3
10	AHCY	82	CNPY2	154	Frizzled-4	226	IL-17C	298	Nrf1	370	ROR1	442	TGF-beta R1
11	AHSG	83	CNTFR	155	Frizzled-5	227	IL-17D	299	OCT3/4	371	RP1	443	TGF-beta R2
12	Akt2	84	COL19A1	156	Frizzled-6	228	IL-19	300	Orexin A	372	RPL12	444	TIMP-1
13	Albumin	85	COTL1	157	Frizzled-7	229	IL-2 R beta	301	OSCAR	373	RPL23A	445	TIMP-2
14	AMPKa1	86	CPE	158	FSTL1	230	IL-24	302	OSM	374	RPLOP	446	Titin
15	ANGPTL2	87	CRADD	159	Galanin	231	IL-27	303	Osteoactivin	375	RPS13	447	TK1
16	ANGPTL3	88	CREB	160	GASP-1	232	IL-28B	304	Osteoadherin	376	RPS14	448	TLR1
17	ANKRD9	89	CRF21	161	GASP-2	233	IL-3	305	Osteoprotegerin	377	RPS15A	449	TLR3
18	ANXA6	90	CRHBP	162	G-CSF R	234	IL-3 R beta	306	p130Cas	378	RPS23	450	TLR4
19	APBA2	91	CrkRS	163	GDF-15	235	IL-5	307	p21	379	RPS3A	451	TMEFF1
20	ApoA1	92	CRTAC1	164	GDF-5	236	IMP2	308	P4HB	380	RPS5	452	TMEFF2
21	ApoA2	93	CRTAM	165	GFRA4	237	INSL3	309	Pappalysin-1	381	RPS8	453	TMEM223
22	ApoB	94	CRTH-2	166	GHR	238	Inulin	310	PCAP	382	RPS9	454	TOMM70A
23	ApoE	95	Cryptin	167	GKN1	239	I-TAC	311	PCPE-1	383	RREB1	455	TPIS
24	ARHGAP1	96	CSE1L	168	GLI-2	240	Jak2	312	PD-1	384	RREB1	456	TPP1
25	ATG5	97	CSK	169	GLIPR2	241	Kallikrein 10	313	PD-ECGF	385	RUSC1	457	TRADD
26	ATPG	98	CTNND1	170	Glut1	242	Kallikrein 11	314	PDGF-AA	386	S100A10	458	TRAIL R2
27	B3GAT1	99	CXCR2	171	Glut2	243	Kallikrein 5	315	PDGF-C	387	S100A11	459	TRAM
28	B4GalT1	100	CXCR4	172	Glut4	244	Kallikrein 6	316	PDGF-D	388	S100A9	460	TRIM14
29	B7-1	101	CXCR7	173	Glut5	245	KIF5B	317	PDGFRB	389	S-100b	461	Tropomyosin 3
30	B7-H2	102	Cyclin D1	174	GM2A	246	LAMA5	318	PDLIM5	390	SBP-1	462	TRRAP
31	BAFF R	103	Cyclophilin F	175	GM-CSF	247	LAMP	319	PDZD2	391	SCF	463	Trypsinogen-2
32	Bax	104	Cystatin A	176	GP2	248	LASP1	320	PENK	392	SCF R	464	TSLP
33	BDNF	105	Cystatin B	177	gp340	249	LBP	321	Pentraxin-3	393	SDF4	465	TSP-1
34	beta-NGF	106	Cystatin D	178	GPD1	250	Lefty-1	322	Perilipin-3	394	Septin-7	466	TSP-2
35	BLAME	107	Cystatin E	179	GPR-39	251	Lefty-A	323	Peroxiredoxin-3	395	SERBP1	467	TSP-4
36	BLMH	108	Cystatin S	180	Granzyme A	252	LHPP	324	PF4	396	Serpin A3	468	TTF1
37	BMP-1	109	DAK	181	Granzyme M	253	LIX	325	PFAS	397	Serpin A5	469	TUBA6
38	BMP-15	110	DCI	182	GRHPR	254	LPS	326	PFDN6	398	Serpin B5	470	TWF2
39	BMP-9	111	DCXR	183	GRP	255	LRG1	327	PHGDH	399	Serpin C1	471	TXNDC15
40	BNIP2	112	DLL4	184	GSK-3 beta	256	LRP-6	328	Piccolo	400	SET	472	TXNDC5
41	BOLA2	113	DMGDH	185	GSN	257	L-Selectin	329	PIK3R2	401	sFRP-4	473	TYRO10
42	BTC	114	DSCAM	186	GSR	258	LUZP1	330	PINCH1	402	SH3BGLR3	474	UBC9
43	BTF3	115	DSG1	187	GSTM1	259	Lymphotactin	331	PIP4K2A	403	SHBG	475	Ubiquitin
44	C1q	116	EDA-A2	188	GSTO1	260	MAdCAM-1	332	PLA2G1B	404	SHOX	476	Ubiquitin+1
45	C1s	117	EDAR	189	GULP1	261	MAN1	333	PLD4	405	Siglec-1	477	UNC45A
46	C3a	118	eEF2	190	HAI-1	262	Mcl-1	334	Plexin B2	406	SLC38A10	478	UNC5H4
47	C5a	119	EG-VEGF	191	Haptoglobin	263	MCP-1	335	PIGF-2	407	SLITRK1	479	uPA
48	CA1	120	eIF4E	192	HB-EGF	264	MCP-5	336	PLS3	408	SLPI	480	UROC1
49	CA2	121	EMAP-II	193	HEG1	265	MDC	337	PNP	409	SLURP1	481	USP2
50	CA3	122	Endothelin	194	Hepassocin	266	MEP1A	338	POMC	410	Smad 1	482	Uteroglobin
51	Calbindin D	123	Eotaxin-2	195	HEXB	267	Mesothelin	339	PON1	411	Smad 4	483	VAP-1
52	Cardiotrophin-1	124	EphA1	196	HGFA	268	MICB	340	PP	412	Smad 5	484	VAP-A
53	Cathepsin A	125	EphA2	197	Histone H2AY	269	MIP-3 alpha	341	PPP1CC	413	Smad 8	485	VARS
54	CCL28	126	EPHX2	198	hnRNPL	270	MIS RII	342	PRAT4B	414	Somatostatin	486	VDAC1
55	CCR3	127	Epregrulin	199	Hoxb3	271	Mitofusin 2	343	PRELP	415	SOX5	487	VEGF
56	CCR4	128	ERRa	200	HOXD11	272	MKK3	344	Prolactin R	416	SPARC	488	VEGF-B
57	CCT3	129	E-Selectin	201	HSP10	273	MKK4	345	ProSAAS	417	SPINK7	489	VEGF-C
58	CD133	130	EVC2	202	HSP47	274	MMP-10	346	Prostasin	418	SPTBN5	490	VEGFR3
59	CD23	131	Factor IX	203	HTRA1	275	MMP-13	347	Protein Z	419	SSTR2	491	VILIP3
60	CD24	132	Factor V	204	HVEM	276	MMP-16	348	Prouroguanylin	420	STXBP2	492	Visfatin
61	CD2AP	133	Factor VII	205	ICAM-1	277	MMP-7	349	PRR4	421	SVEP1	493	Vitronectin
62	CD30	134	Factor XII	206	ICAM-2	278	MRP 1	350	PRRC2A	422	SYK	494	WARS
63	CD40 Ligand	135	FAM3C	207	IDE	279	Multimerin 2	351	PRTN3	423	SYN1	495	WISP-1
64	CD9	136	Fas	208	IFN-beta	280	MuSK	352	P-selectin	424	TACI	496	WISP-2
65	CD90	137	Fas Ligand	209	IFNGR1	281	MyBPC3	353	PSMB1	425	TAGLN2	497	XPD
66	CDC14	138	FGF-11	210	IGFBP-2	282	NACA1	354	PSMD2	426	TALDO	498	XPG
67	CFH	139	FGF-20	211	IGSF4C	283	NADK	355	PSMD9	427	TALDO1	499	YY1
68	CFI	140	FGF-23	212	IL-1 alpha	284	NAGPA	356	PSME1	428	Talin-2	500	ZC3H4
69	CFL1	141	FGF-9	213	IL-1 F10	285	NAPRT1	357	PTHLP	429	TARC		
70	CGA	142	FGF-BP	214	IL-1 F5	286	NeuroD1	358	PTMA	430	TARS		
71	CHCHD3	143	FGFR1	215	IL-1 F6	287	Neurolysin	359	PYY	431	TCA-3		
72	Chemerin	144	FGFR2	216	IL-1 F9	288	Neuropilin-2	360	QARS	432	Tcf20		

VII. Interpretation of Results:

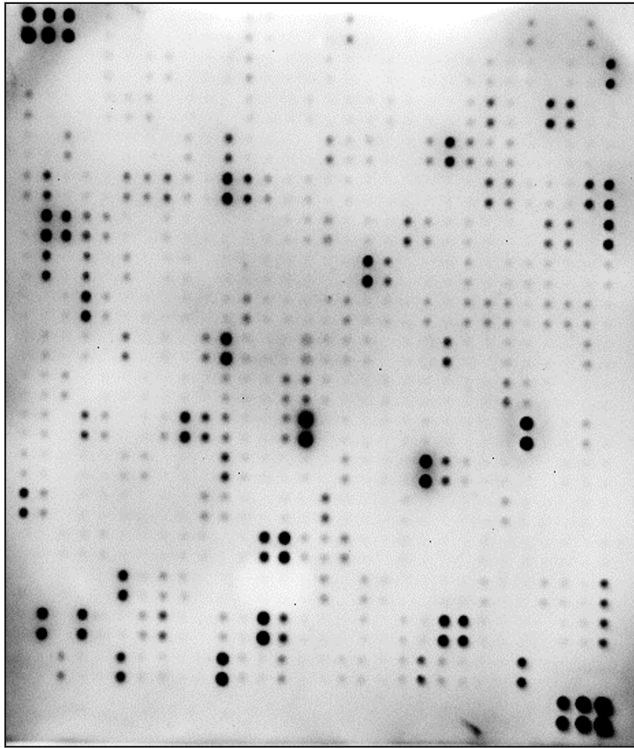
A. Explanation of Controls Spots

To obtain optimal results using a chemiluminescence imaging system (UVP BioImaging Systems), it is suggested to try several different exposure times until the best one is determined. Then, by comparing the signal intensities, relative expression levels of the target proteins can be made. The intensities of signals can be quantified by densitometry. There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized anti-HRP antibodies, which will produce positive control signals after incubation with HRP-conjugated Streptavidin. With all other variables being equal, the Positive Control intensities will be the same for each sub-array, which allows for inter-array normalization. Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies. 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.

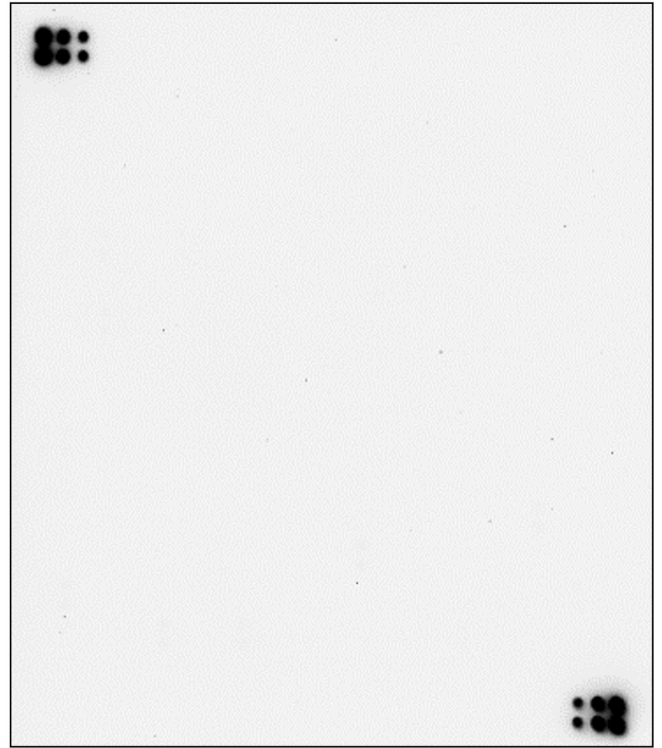
B. Typical Results

The following figure shows the typical result of this array probed with sample(s).

Rat Serum



Buffer Control



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 densitometry data, it is recommended to subtract the local background and normalize to the Positive Control signals before proceeding to analysis.

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.

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 greater than or equal to 1.5-fold increase or less than or equal to 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 is around 95%).

VIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Taking too much time for detection	The whole detection process must be completed within 30 min
	Film developer does not work properly	Fix film developer
	Did not mix HRP-Streptavidin well before use	Mix tube containing HRP-Conjugated Streptavidin well before use since precipitates may form during storage
	Sample is too diluted	Increase sample concentration
	Labeling reagent does not function well	Labeling reagent needs to be saved in -20°C and avoid freeze thaw cycle. Always use fresh labeling reagent for sample labelling.
	Other	
		Slightly increase HRP concentrations
		Work as quickly as possible after mix Detection Buffer C and D
Uneven signal	Bubble formed during incubation	Remove bubbles during incubation
	Membranes were not completely covered with solution	Completely cover membranes with solution
	Insufficient wash	Use more stringent wash
High background	Exposure time is too long	Decrease exposure time
	Membranes dry out during experiment	Completely cover membranes with solution during experiment. Cover tray with lid.
	Sample is too concentrated	Dilute sample

IX. Selected References

Christina Scheel et al., *Paracrine and Autocrine Signals Induce and Maintain Mesenchymal and Stem Cell States in the Breast*. *Cell*. 2011;145, 926-940.

Lin Y, Huang R, Chen L, et al., *Profiling of cytokine expression by biotin-labeled-based protein arrays*. *Proteomics*. 2003, 3: 1750-1757.

Huang R, Jiang W, Yang J, et al., *A Biotin Label-based Antibody Array for High-content Profiling of Protein Expression*. *Cancer Genomics Proteomics*. 2010; 7(3):129-141.

Liu T, Xue R, Dong L, et al., *Rapid determination of serological cytokine biomarkers for hepatitis B-virus-related hepatocellular carcinoma using antibody arrays*. *Acta Biochim Biophys Sin*. 2011; 43(1):45-51.

Cui J, Chen Y, Chou W-C, et al., *An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer*. *Nucl Acids Res*. 2011; 39(4):1197-1207.

Jun Zhong et al., *Temporal Profiling of the Secretome during Adipogenesis in Humans*. *Journal of Proteome Research*. 2010, 9, 5228-5238.

Chowdury UR, Madden BJ, Charlesworth MC, Fautsch MP., *Proteomic Analysis of Human Aqueous Humor*. *Invest Ophthalmol Visual Sci*. 2010; 51(10):4921-4931.

Wei Y, Cui C, Lainscak M, et al., *Type-specific dysregulation of matrix metalloproteinases and their tissue inhibitors in end-stage heart failure patients: relationship between MMP-10 and LV remodeling*. *J Cell Mol Med*. 2011; 15(4):773-782.

Kuranda K, Berthon C, Lepatre F, et al., *Expression of CD34 in hematopoietic cancer cell lines reflects tightly regulated stem/progenitor-like state*. *J Cell Biochem*. 2011; 112(5):1277-1285.

Toh HC, Wang W-W, Chia WK, et al., *Clinical Benefit of Allogenic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients*. *Clin Chem Res*. 2009; 15:7726-7736.

Zhen Hou, *Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosis*. *Biomarkers*. 2009;14(8): 604-618.

Yao Liang Tang, et al., *Hypoxic Preconditioning Enhances the Benefit of Cardiac Progenitor Cell Therapy for Treatment of Myocardial Infarction by Inducing CXCR4*.

Circ Res. 2009;109:197723.

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