RayBio® Label-Based (L-Series) Rabbit L1 Array, Membrane

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAL-BLM-1-2 (2 Sample Kit)
AAL-BLM-1-4 (4 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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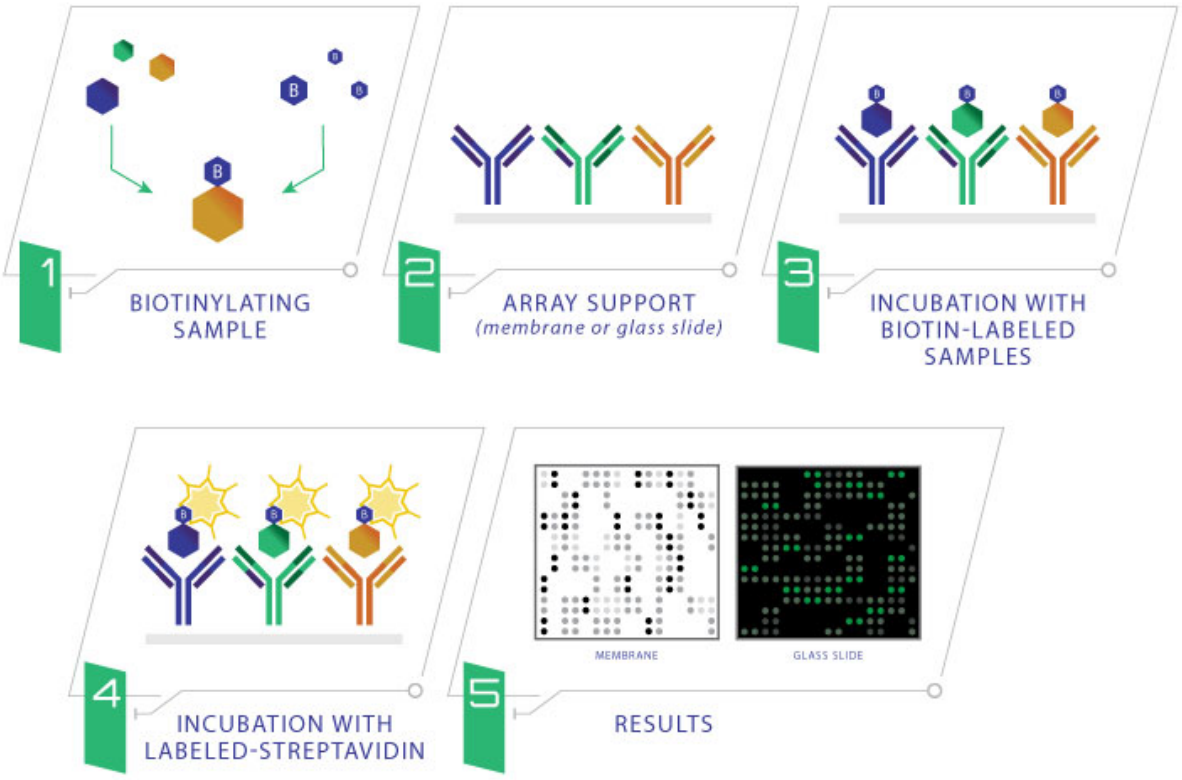
I. Introduction

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-Omat™ 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 $1x10^6$ 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.**,+
    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 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. 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 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.

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).

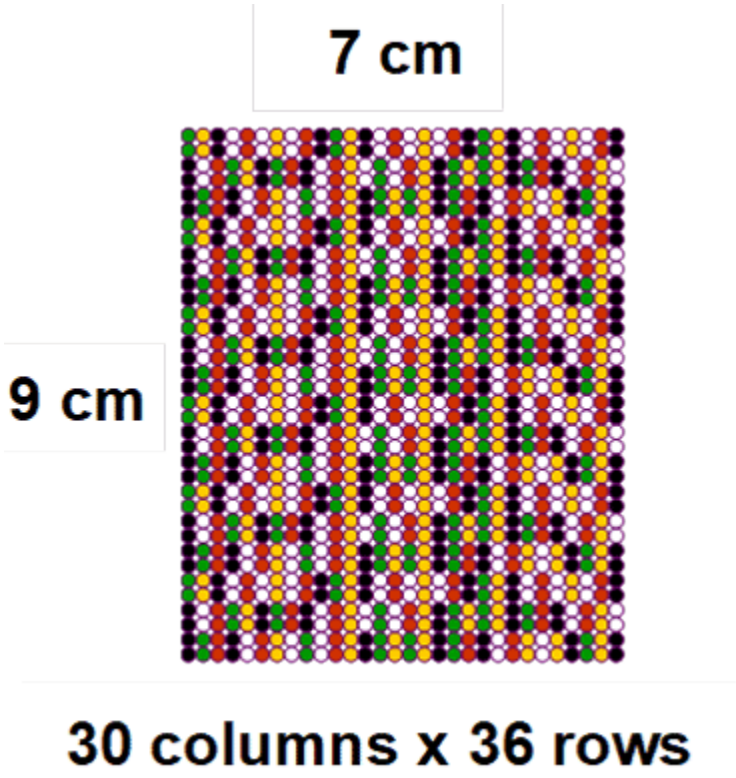
B. Handling the Array Membranes

- 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.

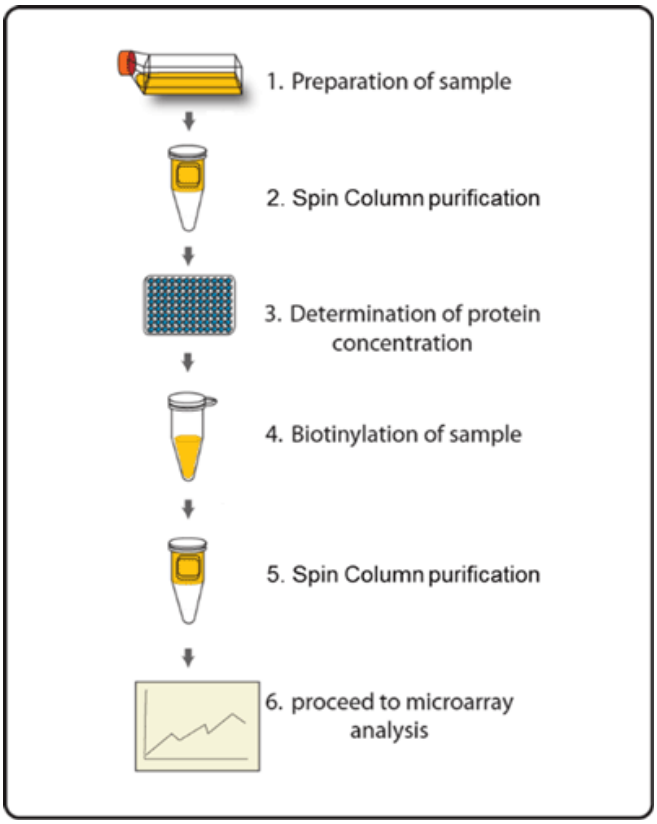
D. Layout of Array Membrane



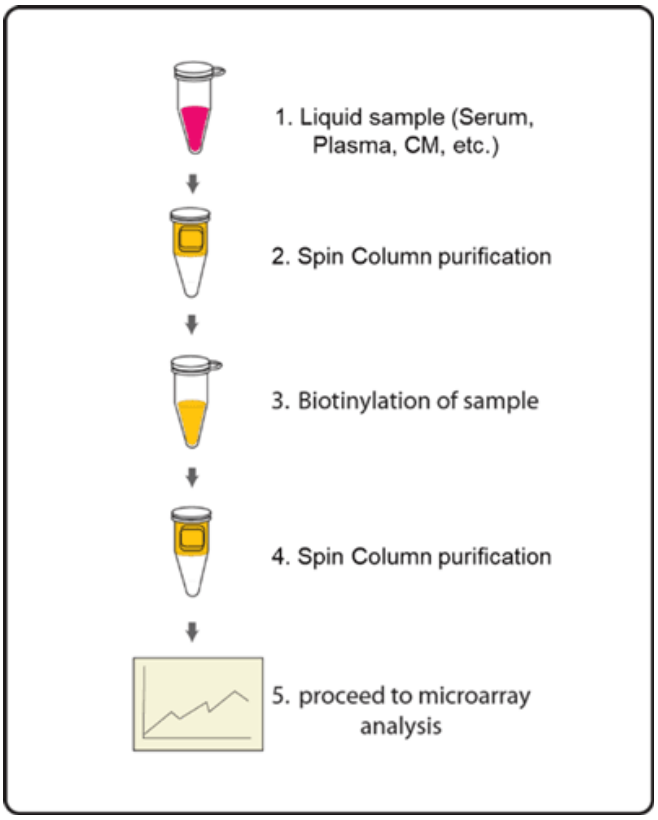
IV. Protocol

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 µl neat supernatant*
- *Serum/Plasma: 10 µl serum/plasma in 600 µl Labeling Buffer*
- *Cell/tissue lysate: 100 µg lysate in 500 µl Labeling Buffer*

Note: The maximal sample volume is 700 µl for each Spin Column. Do not load over 700 µl of sample into a Spin Column.

B. Biotin-Labeling the Sample

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

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.
6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.
    a. For labeling cell culture supernatants: Add 10 µl of Labeling Reagent into the sample tube (for 600 µl supernatant).
    b. For labeling serum or plasma: Add 10 µl of Labeling Reagent into the sample tube (for 10 µl serum/plasma *in 600 µl labeling buffer*).
    c. For labeling cell or tissue lysates: Add 5 µl of 1X Labeling Reagent into the sample tube (for 100 µg lysate *in 500 µl labeling buffer*).
    d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

    Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If more or less amount sample is labelled, adjust this volume proportionally.
7. Add 5 µl Stop Solution (Item D) to each sample tube. Using a new spin column, repeat Steps 1-4 of section A. Sample Purification to remove the

excess non-reacted biotin reagent from each sample.

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

C. 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
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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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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	11b-HSD1	73	CD30	145	EVC2	217	Histone H1.3	289	LECT2	361	PDX-1	433	SPARC
2	4-1BB	74	CD36	146	EXTL2	218	Histone H3.3	290	LEDGF	362	PEBP1	434	SP-D
3	6Ckine	75	CD38	147	FABP4	219	HMGB1	291	Leptin	363	Pentraxin-3	435	Src
4	ACE-2	76	CD90	148	Factor IX	220	hnRNPL	292	Leptin R	364	Periostin	436	SREC-II
5	ACTC1	77	CD97	149	Factor VII	221	hnRNPU	293	LIF	365	PF4	437	SSEA-1
6	Activin R1B	78	CD98	150	Factor XI	222	HOXB3	294	LIF R alpha	366	PFAS	438	SSTR2
7	Activin R2A	79	CDSN	151	Factor XII	223	HOXD11	295	Lipocalin-2	367	PGM1	439	SVEP1
8	ADAMTS1	80	Cerberus 1	152	FAM3C	224	HPRT	296	LIX	368	PGRP-S	440	TCP1 eta
9	ADAMTS15	81	CFHR5	153	Fas Ligand	225	HRG	297	LRP-6	369	PHGDH	441	TFF1
10	ADAMTS4	82	Chemerin	154	FASN	226	HRG-beta 1	298	LSAMP	370	PLA2G1B	442	TGF beta 3
11	ADAMTS5	83	Chitotriosidase	155	FGF Basic	227	HSP10	299	L-Selectin	371	Plasminogen	443	TGF beta R2
12	Adiponectin	84	Chymase	156	FGF R4	228	HSP90	300	LTB	372	PLOD2	444	TGF beta R3
13	Aggrecan	85	CKB	157	FGF-10	229	HTRA1	301	LTBR	373	PNP	445	TGF-alpha
14	AHCY	86	CKMB	158	FGF-11	230	HTRA2	302	LYVE-1	374	Podocalyxin	446	TGF-beta 2
15	AK1	87	Claudin-4	159	FGF-16	231	ICAM-1	303	Matrilin-3	375	POR	447	TGF-beta R1
16	ALDH1A1	88	CLC	160	FGF-19	232	ICAM-2	304	Mcl-1	376	PP	448	Thioredoxin-1
17	ALK	89	CMASP3	161	FGF-21	233	ICAM-5	305	M-CSF	377	PRCP	449	Thrombopoietin
18	Alpha 1 AG	90	COL19A1	162	FGF-23	234	IDH1	306	MDC	378	Pref-1	450	TIM-4
19	alpha-Synuclein	91	COL1A1	163	FGF-9	235	IFNAR1	307	Mer	379	PREP	451	TIMP-1
20	AMPKa1	92	Contactin-1	164	FGFR1 alpha	236	IFNg	308	Metavinculin	380	Presenilin 1	452	TIMP-2
21	AMSH	93	Contactin-4	165	FGFR2	237	IFNGR1	309	MFRP	381	Procalcitonin	453	TL1A
22	ANG-1	94	Cortactin	166	FGL2	238	IGF-2	310	MIP-1 beta	382	Prolactin	454	TLR1
23	ANG-2	95	CPA1	167	Fibronectin	239	IGFBP-1	311	MIP-3 beta	383	Prolargin	455	TLR2
24	Angiogenin	96	CRHBP	168	Fibulin 3	240	IGFBP-6	312	MMP-10	384	Prostasin	456	TLR3
25	ANGPTL2	97	CRTAC1	169	FKBP12	241	IL-12RB2	313	MMP-13	385	Protein C	457	TLR4
26	ANGPTL3	98	CRTAM	170	FKBP25	242	IL-13	314	MMP-16	386	PSMA1	458	TMEFF2
27	Annexin A5	99	CT-1	171	FKBP51	243	IL-16	315	MMP-2	387	PTEN	459	TNFa
28	Annexin A7	100	CTLA-4	172	FLRG	244	IL-17A	316	MMP-7	388	PTH	460	TOP2B
29	ApoA1	101	CXCL3	173	Follistatin	245	IL-17F	317	MMP-8	389	PTP gamma	461	TPP1
30	ApoA2	102	CXCL9	174	Frizzled-4	246	IL-17RA	318	MMP-9	390	PTP mu	462	TRADD
31	ApoB	103	CXCR1	175	Frizzled-6	247	IL-17RB	319	MP1	391	PTPRZ	463	TRANCE
32	Artemin	104	CXCR2	176	FSH	248	IL-17RD	320	MPO	392	RANTES	464	TRAP220
33	B3GNT1	105	CXCR3	177	FSTL1	249	IL-19	321	MRP 1	393	Ras	465	TROY
34	Bax	106	CXCR4	178	FUCA1	250	IL-1a	322	MSH6	394	RBP4	466	TSG-6
35	beta-I Tubulin	107	CXCR5	179	G6PD	251	IL-1b	323	MyBPC3	395	Reg1A	467	TSH
36	BLAME	108	CXCR6	180	Galectin-3	252	IL-1F6	324	Myoglobin	396	RELT	468	TSP-1
37	BLC	109	Cyclin D1	181	GALNT2	253	IL-1R1	325	NCAM-1	397	Resistin	469	TSP-2
38	BLMH	110	Cyclophilin B	182	GASP-1	254	IL-1R2	326	Nectin-3	398	ROBO4	470	TSP-4
39	BMP-1	111	Cystatin A	183	Gastrokine 1	255	IL-1R3	327	NET1	399	ROCK2	471	TWEAK
40	BMP-2	112	Cystatin B	184	GCSF	256	IL-1R4	328	Netrin-4	400	RREB1	472	TWEAK R
41	BMP-3	113	Cystatin C	185	GCSF R	257	IL-1R6	329	Neurokinin A	401	S100A1	473	TXNDC15
42	BMP-4	114	Cytochrome C	186	GDF5	258	IL-1RA	330	Neuropilin-1	402	S100A10	474	TXNDC5
43	BMP-6	115	DAN	187	Gephyrin	259	IL-2	331	Neuropilin-2	403	S100A11	475	Ubiquitin+1
44	BMP-9	116	DDAH1	188	GFR alpha-1	260	IL-20	332	Neurotrimin	404	S100A9	476	UCH-L1
45	BMPR-IB	117	D-Dimer	189	GFR alpha-2	261	IL-20RB	333	Nidogen-2	405	SCF	477	UNC5H4
46	BMPR-II	118	Decorin	190	GFR alpha-3	262	IL-21	334	Notch-1	406	SCF R	478	uPA
47	BRCA2	119	Desmocollin-2	191	GHR	263	IL-22	335	Notch-3	407	SCGF	479	uPAR
48	C1QA	120	Desmocollin-3	192	GLI-2	264	IL-22BP	336	NPTXR	408	Serpin A12	480	UROC1
49	C1QC	121	Desmoglein-2	193	GLIPR2	265	IL-23	337	NR3C3	409	Serpin A3	481	UROD
50	C3a	122	DISC 1	194	GLO-1	266	IL-23R	338	NrCAM	410	Serpin B4	482	USP14
51	C5a	123	DKK-1	195	Glut2	267	IL-26	339	OBCAM	411	Serpin B5	483	USP2
52	CA1	124	Dystroglycan	196	Glut3	268	IL-27RA	340	Olfactomedin-1	412	Serpin B8	484	Uteroglobin
53	CA3	125	E-Cadherin	197	Glut5	269	IL-4	341	OSCAR	413	Serpin C1	485	VAP-1
54	CA9	126	EDA-A2	198	Glycoprotein V	270	IL-6	342	OSM R beta	414	Serpin D1	486	VARS
55	Cadherin-6	127	EDG-1	199	Glypican 3	271	IL-7	343	Osteoactivin	415	Serpin F1	487	VE-Cadherin
56	CALD1	128	EG-VEGF	200	GM-CSF	272	IL-7RA	344	Osteoadherin	416	Serpin F2	488	VEGF-A
57	CapG	129	Endocan	201	GMF beta	273	IL-8	345	Osteopontin	417	SerRS	489	VEGF-C
58	Caspase-14	130	Endoglin	202	gp340	274	IL-9R	346	Osteoprotegerin	418	sFRP-4	490	VEGFR1
59	Catalase	131	Endorepellin	203	GPD1	275	ILK	347	OX40 Ligand	419	SHP-1	491	VEGFR2
60	Cathepsin A	132	EN-RAGE	204	GPLD1	276	INSRR	348	P20Sb3	420	Siglec-1	492	VEGFR3
61	Cathepsin B	133	Eotaxin-1	205	GPR-39	277	Insulysin	349	p27	421	SLITRK1	493	Versican
62	Cathepsin G	134	EpCAM	206	Grb2	278	Integrin beta 6	350	P4HB	422	Smad 1	494	VILIP3
63	CCL3	135	EPCR	207	GRP75	279	IP-10	351	p53	423	Smad 4	495	Vimentin B
64	CCR1	136	EphA1	208	GRP78	280	I-TAC	352	PAK7	424	Smad 5	496	Vitronectin
65	CCR6	137	EphA2	209	H6PD	281	Kallikrein 5	353	PCNA	425	Smad 7	497	VSIG4
66	CCR7	138	EphB6	210	HABP2	282	Kallikrein 7	354	PCPE-1	426	SMPD4	498	WISP-1
67	CCR8	139	Ephrin-A1	211	HAI-1	283	Kallikrein 8	355	PCSK9	427	SOD2	499	XIAP
68	CCR9	140	Epigen	212	Haptoglobin	284	KLKB1	356	PDAP1	428	SOD3	500	ZC3H4
69	CD109	141	Epiregulin	213	HBB	285	KPNB1	357	PDGF R beta	429	Somatostatin		
70	CD133	142	EPOR	214	HB-EGF	286	LAMB2	358	PDGF-BB	430	SorLA		
71	CD157	143	ErbB2	215	CGA	287	Layilin	359	PDGF-C	431	SOST		
72	CD23	144	ErbB4	216	HEXB	288	LBP	360	PD-L1	432	SOX2		

VII. Interpretation of Results:

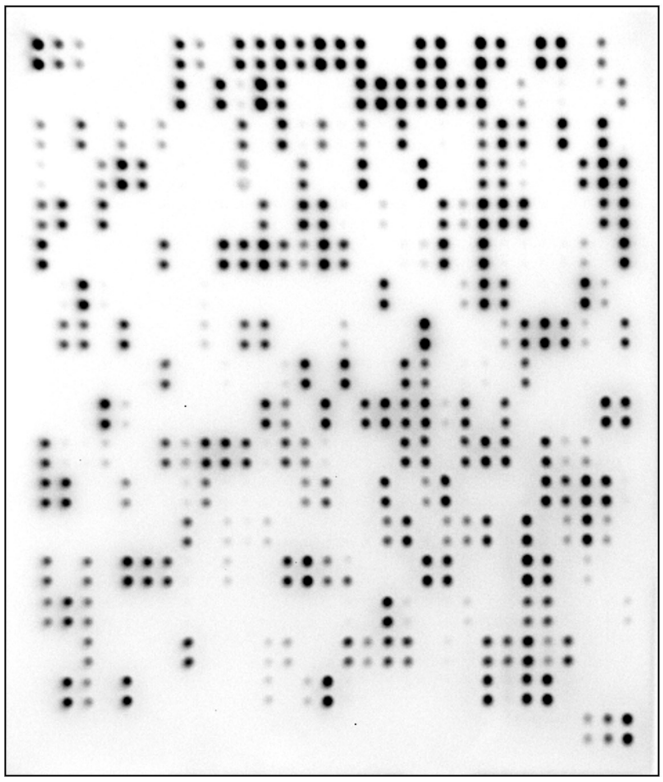
A. Explanation of Controls Spots

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.

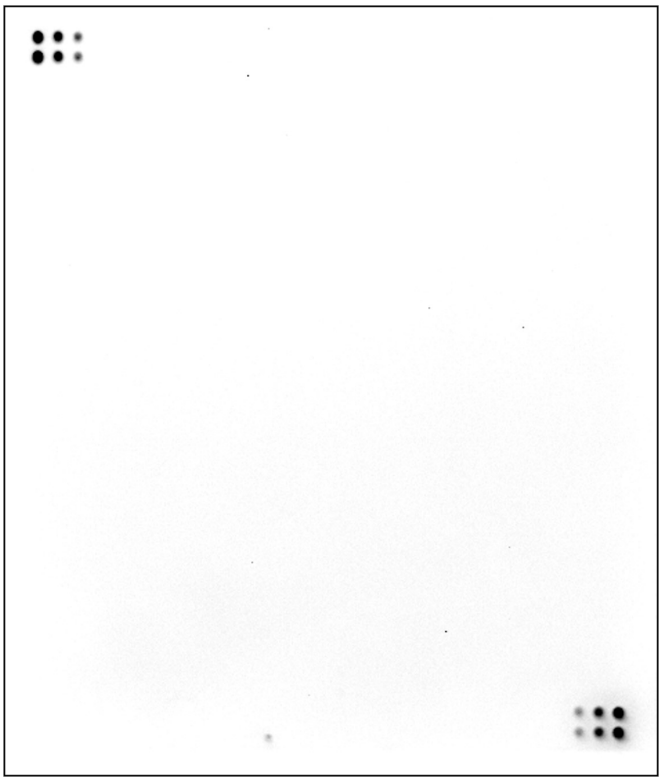
B. Typical Results

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

Rabbit 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	Check if there were any contamination with any solution containing amines in biotin-labeling step
		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

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