RayBio® Label-Based (L-Series) Human L493 Array Membrane Kit

Patent Pending Technology
User Manual (Jan 1, 2022)

For the simultaneous detection of the relative expression of 493 Human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Human L493 Array, Membrane
AAH-BLM-2-2 (2 Sample Kit)
AAH-BLM-2-4 (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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Tel: (Toll Free) 1-888-494-8555 or +1-770-729-2992; Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

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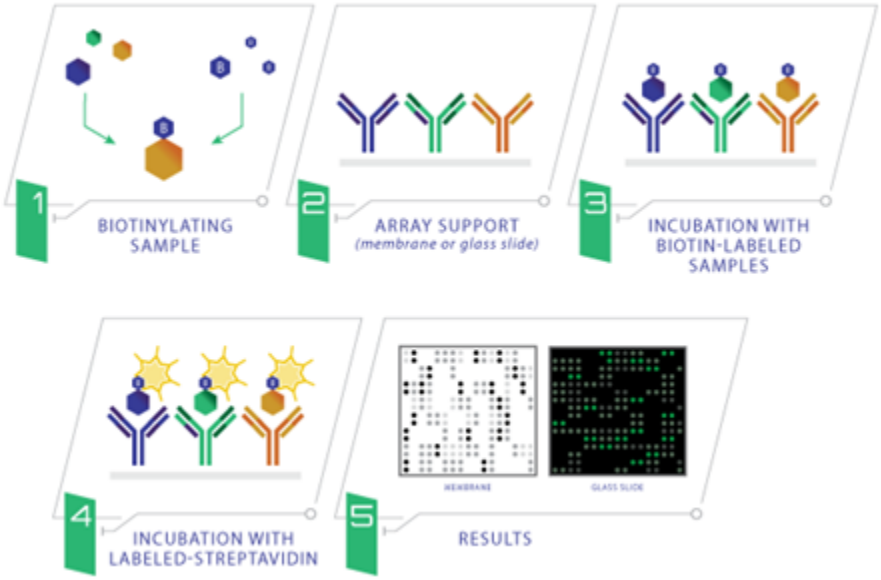
I. Introduction

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).

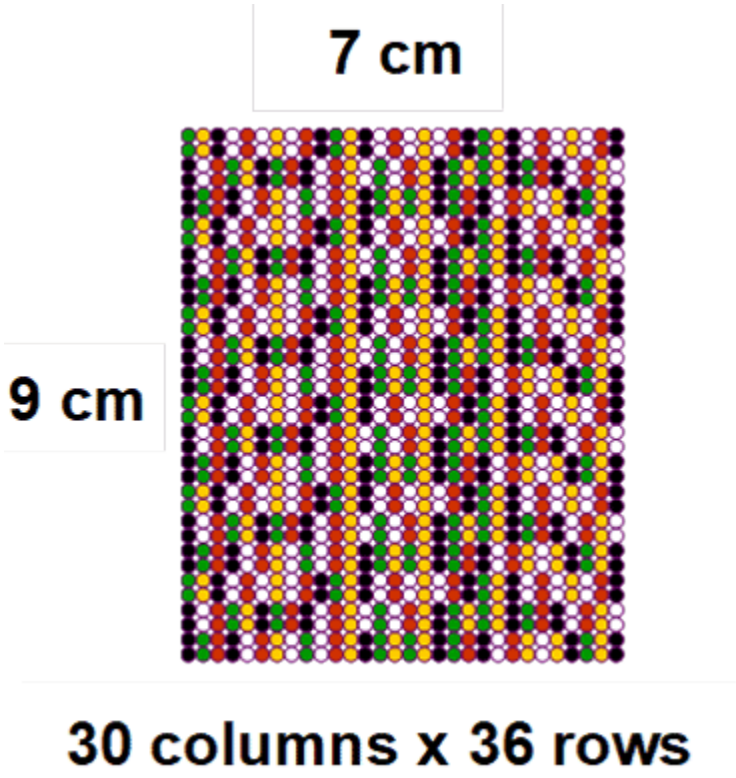
B. Handling the Array Membranes

- 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.

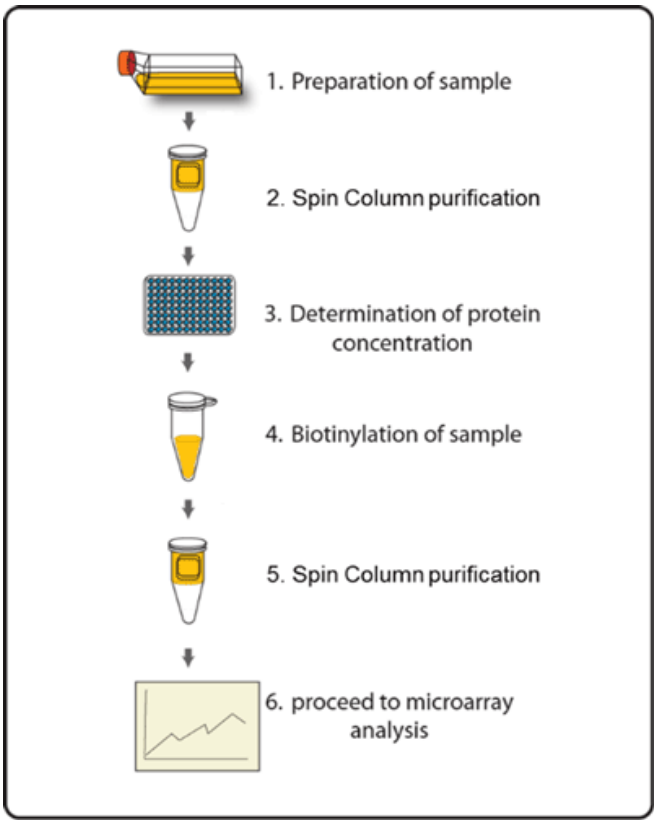
D. Layout of Array Membrane



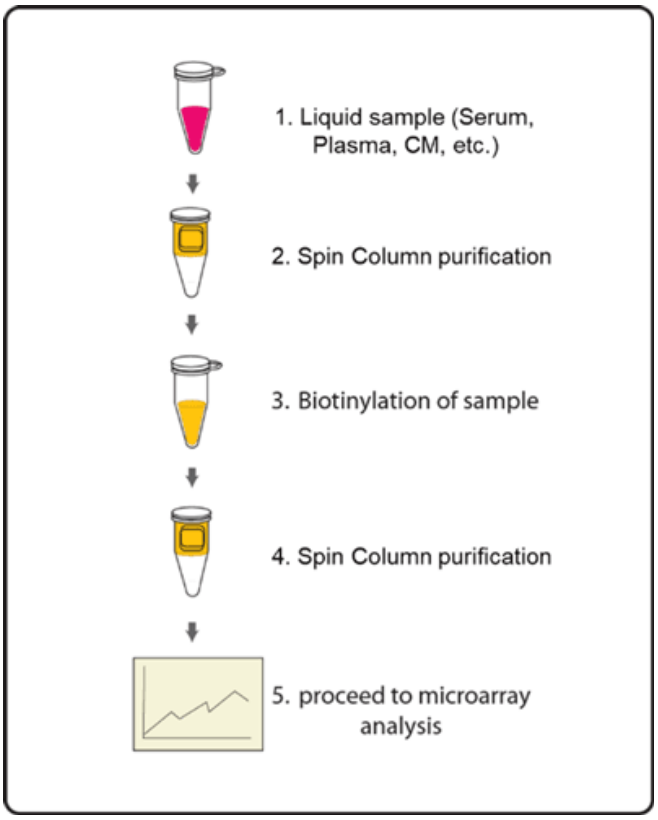
IV. Protocol

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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36	Blank	Blank	Blank	Blank	486	487	488	489	490	491	492	493	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	11b-HSD1	73	BMX	145	CRTAM	217	FRK	289	KLF4	361	PI 16	433	SCGF
2	2B4	74	BNIP2	146	CSH1	218	ARB1	290	LAG-3	362	PIK3R1	434	SOST
3	4-1BB	75	BNP	147	Troponin T	219	Furin	291	Layilin	363	PIM2	435	SOX17
4	A1BG	76	Btk	148	CutA	220	Fyn	292	LDL R	364	PKM2	436	SOX2
5	A2M	77	C2	149	Cyclin D1	221	GADD45A	293	Legumain	365	Plasminogen	437	SPARCL1
6	ABL1	78	C3a	150	Cystatin A	222	Galanin	294	LH	366	Podocalyxin	438	SPINK1
7	ACE	79	C5a	151	Cystatin B	223	Galectin-1	295	LIMPII	367	POMC	439	SRMS
8	ACE-2	80	C7	152	Cystatin C	224	Galectin-3BP	296	LIN41	368	PON1	440	SSEA-1
9	ACK1	81	C8b	153	Cytochrome C	225	Galectin-7	297	Livin	369	PON2	441	SSEA-4
10	ACPP	82	C9	154	Cytokeratin 8	226	gamma-Thrombin	298	LOX-1	370	PPARg2	442	SSTR2
11	ACTH	83	CA9	155	Cytokeratin 18	227	Gas1	299	LPS	371	PPP2R5C	443	SSTR5
12	ADAM-9	84	CA15-3	156	Cytokeratin 19	228	Gastrin	300	LRG1	372	Presenilin 1	444	Survivin
13	ADAMTS1	85	CA19-9	157	DBI	229	GATA-3	301	LTF	373	Presenilin 2	445	SYK
14	ADAMTS10	86	CA125	158	DCBLD2	230	GATA-4	302	LTK	374	Pro-BDNF	446	Syndecan-1
15	ADAMTS13	87	Cadherin-13	159	D-Dimer	231	Gelsolin	303	Lumican	375	Procalcitonin	447	Syndecan-3
16	ADAMTS15	88	CLEC14A	160	DEFA1/3	232	Ghrelin	304	Lyn	376	Pro-Cathepsin B	448	TACE
17	ADAMTS17	89	Calbindin D	161	CPA1	233	GLP-1	305	LYRIC	377	Progesterone	449	TAF4
18	ADAMTS18	90	Calcitonin	162	Desmin	234	GMNN	306	LYVE-1	378	pro-Glucagon	450	Tec
19	ADAMTS19	91	Calreticulin	163	DLL1	235	GPBB	307	LZTS1	379	Prohibitin	451	TFF1
20	ADAMTS4	92	Calsyntenin-1	164	DLL4	236	GPI	308	Mammaglobin A	380	Pro-MMP-7	452	TFF3
21	ADAMTS5	93	CART	165	DMP-1	237	GPR-39	309	Marapsin	381	Pro-MMP-9	453	Thrombin
22	ADAMTSL2	94	Caspase-3	166	DPPIV	238	GPX1	310	MATK	382	Pro-MMP-13	454	Thrombomodulin
23	Adipsin	95	Caspase-8	167	E-Cadherin	239	GPX3	311	MBL	383	ProSAAS	455	TK1
24	Afamin	96	Cathepsin B	168	Endorphin Beta	240	GRP	312	C1qTNF1	384	Prostasin	456	Thyroglobulin
25	AFP	97	Cathepsin D	169	EDNRA	241	GRP75	313	Mer	385	Protein p65	457	TIM-1
26	ALBUMIN	98	Cathepsin L	170	Enolase 2	242	GRP78	314	Mesothelin	386	PSA-Free	458	TNK1
27	Aldolase A	99	Cathepsin S	171	ENPP2	243	GSR	315	MICB	387	PSA-total	459	TOPORS
28	Aldolase B	100	CBP	172	EpCAM	244	GST	316	Midkine	388	PSP	460	TPA
29	Aldolase C	101	CCK	173	EphA1	245	HADHA	317	MINA	389	PTH	461	TPM1
30	ALK	102	CD23	174	EphA2	246	HAI-1	318	MSHa	390	PTHLP	462	TRA-1-60
31	Alpha 1 AG	103	CD24	175	EphA3	247	HAI-2	319	MTUS1	391	PTN	463	TRA-1-81
32	A1M	104	CD36	176	EphA4	248	Haptoglobin	320	Myoglobin	392	PTPRD	464	Transferrin
33	Alpha Lactalbumin	105	CD38	177	EphA5	249	hCG alpha	321	NAIP	393	PYK2	465	Trappin-2
34	ALPP	106	CD44	178	EphA6	250	hCGb	322	Nanog	394	PYY	466	TRKB
35	AMICA	107	CD45	179	EphA7	251	Hck	323	NELL2	395	Ras	467	Troponin I
36	AMPKa1	108	CD46	180	EphA8	252	HE4	324	Neprilysin	396	RBP4	468	Troponin C
37	Amylin	109	CD47	181	EphB1	253	Hemopexin	325	Nesfatin	397	RECK	469	TRPC1
38	ANGPTL3	110	CD55	182	EphB2	254	Hepcidin	326	Nestin	398	RELM alpha	470	TRPC6
39	ANGPTL4	111	CD59	183	EphB3	255	HOXA10	327	NET1	399	Resistin	471	TRPM7
40	Annexin A7	112	CD61	184	EphB4	256	HSP10	328	Netrin G2	400	RET	472	Trypsin 1
41	APC	113	CD71	185	EphB6	257	HSP20	329	Netrin-4	401	RIP1	473	TSH
42	APCS	114	CD74	186	ERRa	258	HSP27	330	Neurokinin A	402	ROCK1	474	TSLP
43	Apelin	115	CD79 alpha	187	Erythropoietin R	259	HSP32	331	Neuropeptide Y	403	ROCK2	475	TXK
44	Apex1	116	CD90	188	ESAM	260	HSP40	332	NF1	404	ROR1	476	Tyk2
45	APN	117	CD97	189	EV15L	261	HSP60	333	NM23-H1/H2	405	ROR2	477	TYRO10
46	ApoA1	118	CD200	190	EXTL2	262	HSP70	334	Notch-1	406	ROS	478	Uromodulin
47	ApoA2	119	CEA	191	FABP1	263	HSP90	335	NPTX1	407	RYK	479	Vasopressin
48	ApoA4	120	CEACAM-1	192	FABP2	264	HSPA8	336	NPTXR	408	S100A4	480	VDUP-1
49	ApoB	121	Ceruloplasmin	193	FABP3	265	HTRA2	337	NR3C3	409	S100A6	481	VEGF R1
50	ApoB100	122	CFHR2	194	FABP4	266	IBSP	338	Ntn1	410	S100A8	482	VGF
51	ApoC1	123	Chemerin	195	Fc gamma RIIIB	267	IGF2BP1	339	OCT3/4	411	S-100b	483	VIPR2
52	ApoC2	124	CHI3L1	196	Factor XIII B	268	IGFBP-5	340	Omentin	412	SART1	484	Visfatin
53	ApoC3	125	Chromogranin A	197	FAK	269	IDUA	341	Osteocalcin	413	SART3	485	VDR
54	ApoD	126	Chymase	198	FAP	270	IL-33	342	Osteopontin	414	SCG3	486	VDB
55	ApoE	127	cIAP-2	199	Fcg RIIB/C	271	IL-34	343	OX40	415	Selenoprotein P	487	PROS1
56	ApoE3	128	Ck beta 8-1	200	Fen-1	272	IL-28B	344	p21	416	SEMA3A	488	Vitronectin
57	ApoH	129	CKMB	201	FER	273	INSL3	345	p27	417	Serotonin	489	VWF
58	ApoM	130	Claudin-3	202	Ferritin	274	INSRR	346	p53	418	Serpin G1	490	WT1
59	APP	131	Claudin-4	203	Fetuin A	275	ITGAV	347	PAI-1	419	Serpin A1	491	XIAP
60	ASPH	132	CLEC3B	204	Fetuin B	276	Itk	348	PAK7	420	Serpin A3	492	ZAG
61	Attractin	133	Clusterin	205	FGFR1	277	ITM2B	349	Pancreastatin	421	Serpin A4	493	ZAP70
62	B3GNT1	134	CNDP1	206	FGFR1 alpha	278	Kallikrein 2	350	PP	422	Serpin A5		
63	BAF57	135	COCO	207	FGFR2	279	Kallikrein 5	351	Pappalysin-1	423	Serpin A8		
64	BAFF	136	CFH	208	Fibrinogen	280	Kallikrein 6	352	PARK7	424	Serpin A9		
65	BAI-1	137	Contactin-1	209	Fibrinopeptide A	281	Kallikrein 7	353	P-Cadherin	425	Serpin A12		
66	BCAM	138	Contactin-2	210	Fibronectin	282	Kallikrein 8	354	PCAF	426	Serpin B5		
67	B2M	139	CBG	211	Ficolin-3	283	Kallikrein 10	355	PD-1	427	Serpin D1		
68	Beta Defensin 4	140	COX-2	212	FIH	284	Kallikrein 11	356	PDX-1	428	Serpin I1		
69	Beta IG-H3	141	C-peptide	213	FOLR1	285	Kallikrein 14	357	PEDF	429	SERTAD2		
70	Biglycan	142	CPN2	214	FOXN3	286	KCC3	358	PEPSINOGEN I	430	SHBG		
71	BLAME	143	Creatinine	215	FoxO1	287	KCTD10	359	PEPSINOGEN II	431	SMAC		
72	BMP-9	144	CRP	216	FoxP3	288	KIF3B	360	PGRP-S	432	SNCG		

VII. Interpretation of Results:

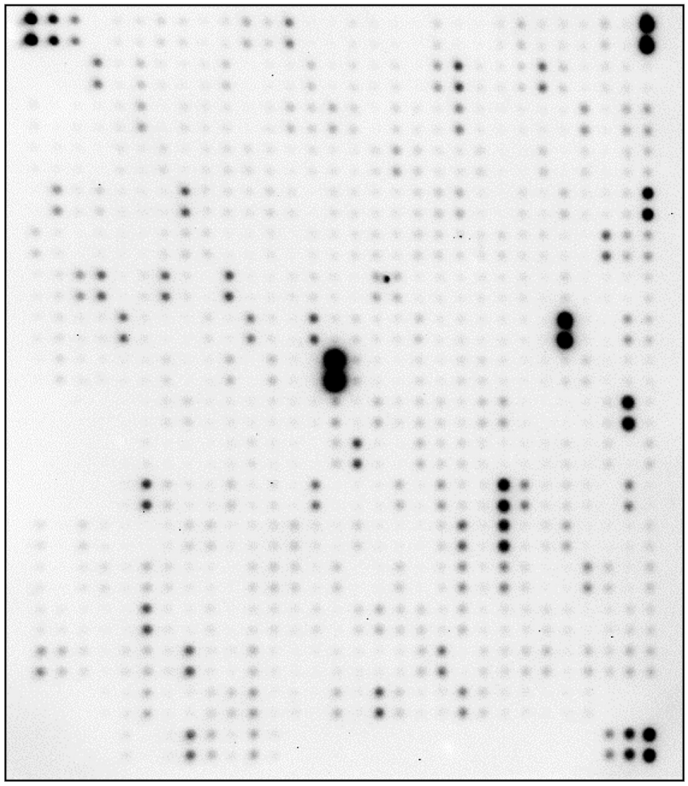
A. Explanation of Controls Spots

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.

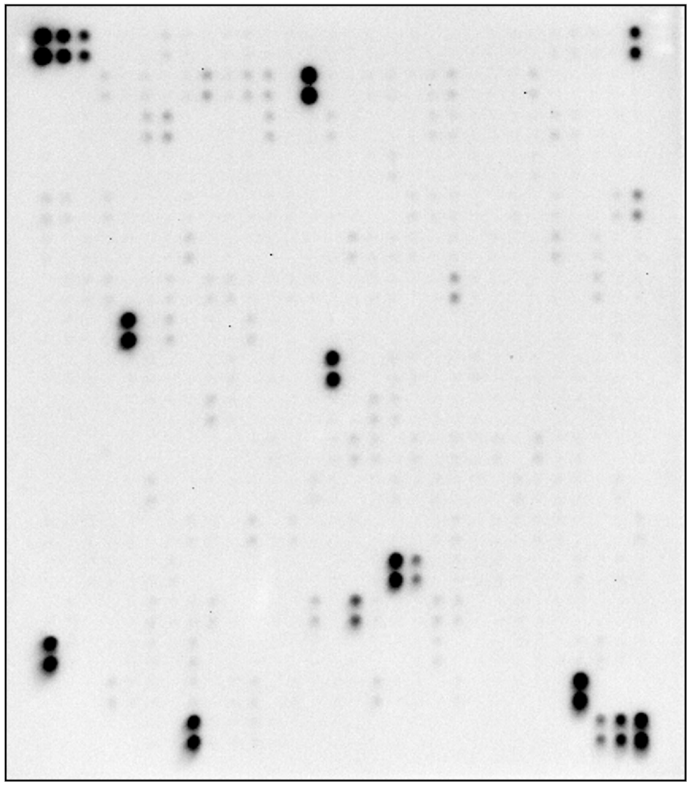
B. Typical Results

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

Human Serum



Sample B



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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