RayBio® Label-Based (L-Series) Human L5 Array, Membrane

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAH-BLM-5-2 (2 Sample Kit)
AAH-BLM-5-4 (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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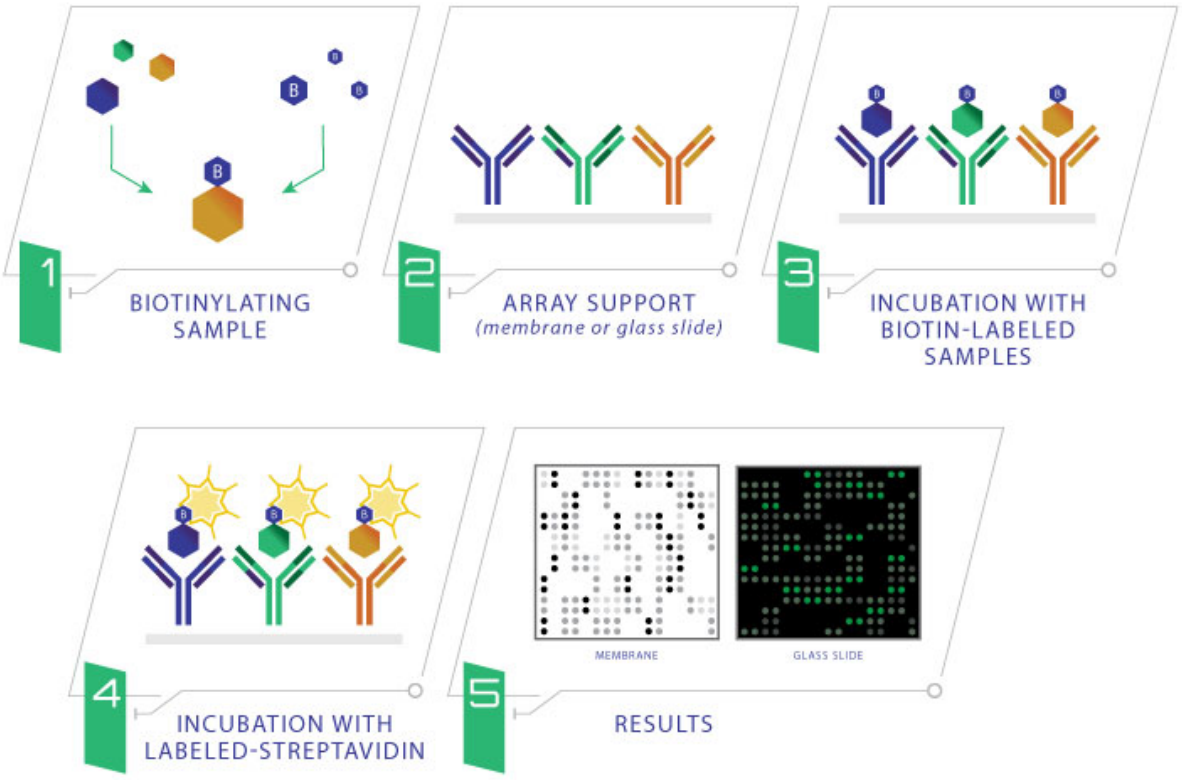
I. Introduction

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).

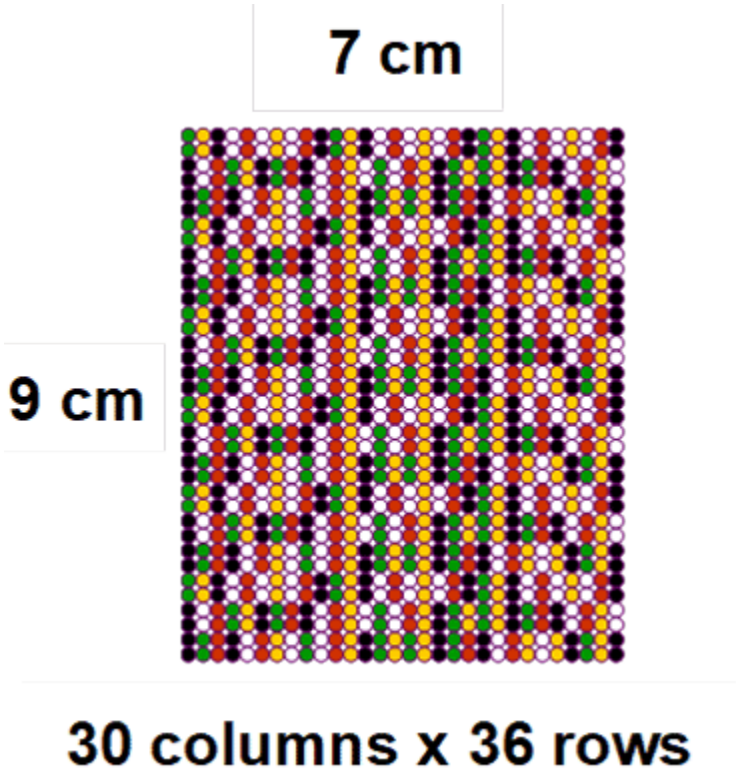
B. Handling the Array Membranes

- 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.

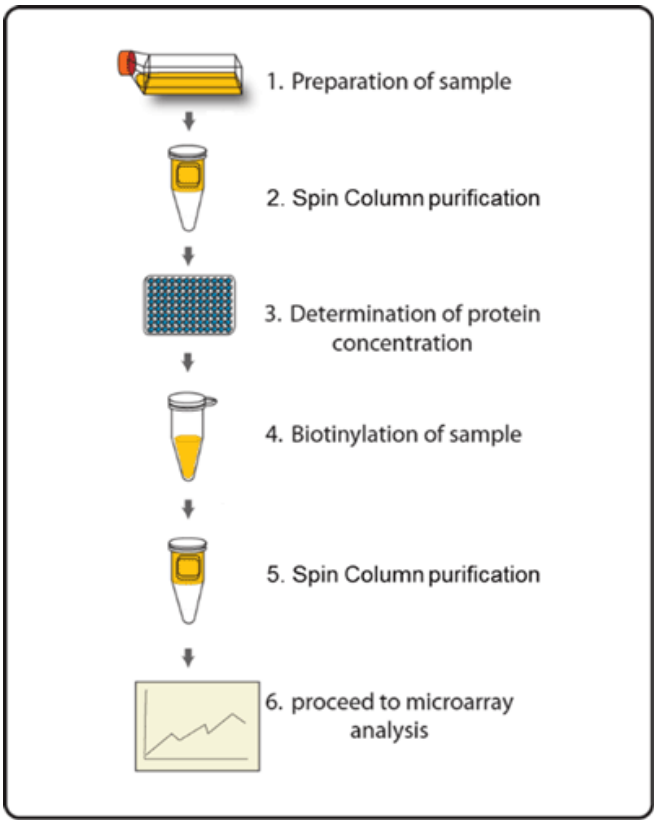
D. Layout of Array Membrane



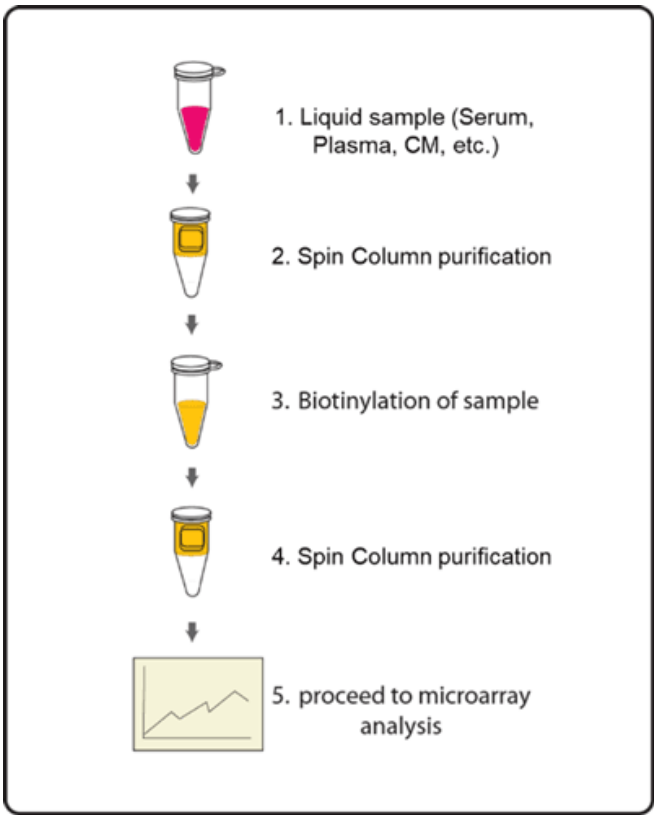
IV. Protocol

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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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	A4GNT	73	CLDN1	145	F2RL1	217	ITGB8	289	NETO2	361	PSMD4	433	SPRED2
2	AAK1	74	CLDN10	146	FAM123B	218	ITLN2	290	NEUROD2	362	PTCH1	434	SPRY2
3	ABI1	75	CLDN12	147	FERMT2	219	ITM2A	291	NFAM1	363	PTGER1	435	SPRYD4
4	ACKR1	76	CLDN15	148	FLI1	220	ITM2C	292	NFATC1	364	PTGER2	436	SPTBN2
5	ADAM33	77	CLDN17	149	FOS	221	KDM4A	293	NKX2-1	365	PTGER3	437	SRPK2
6	ADAMTS3	78	CLDN19	150	FOXC1	222	KDM4C	294	NKX3-1	366	PTK6	438	STMN2
7	ADAMTS8	79	CLDN6	151	FOXD3	223	KDM5B	295	NME2	367	PTPN14	439	STXBP2
8	ADGRA2	80	CLTCL1	152	FOXF2	224	KHDRBS1	296	NNMT	368	PTPRN	440	STXBP3
9	ADGRE3	81	CNMD	153	FOXJ3	225	KIAA1303	297	NOD1	369	PTPRT	441	SULF2
10	ADGRG3	82	COMMD1	154	FOXK1	226	KLF12	298	NOMO1	370	PYCARD	442	SUSD2
11	ADIPOR1	83	COPS5	155	FOXL2	227	KLF2	299	NR1D2	371	PYGO1	443	SVEP1
12	ADNP	84	COX4I1	156	FOXP2	228	KLF5	300	NR1I3	372	RACGAP1	444	SVIL
13	ADORA2A	85	COX4I2	157	FOXP4	229	KLF6	301	NR2C1	373	RAP2B	445	SWAP70
14	ADRB1	86	CRNN	158	FRAT2	230	KPNA1	302	NR2C2	374	RARA	446	SYBU
15	ADRBK2	87	CRTC2	159	FXYD5	231	KPNA4	303	NR2E1	375	RARB	447	SYN1
16	AFAP1	88	CRTC3	160	GAB2	232	LEF1	304	NR2E3	376	RARG	448	SYP
17	AGR2	89	CRX	161	GABBR1	233	LGALS12	305	NR2F6	377	RARRES1	449	TAP2
18	AGTR1	90	CSRP2	162	GAK	234	LGI2	306	NR4A3	378	RB1	450	TBK1
19	AICDA	91	CTBP1	163	GALNT4	235	LHX1	307	NUB1	379	RBPJ	451	TBX5
20	AIRE	92	CTCF	164	GAPDHS	236	LIPI	308	NXN	380	RELB	452	TCF7
21	ANXA10	93	CUX1	165	GBX2	237	LMO4	309	OAS2	381	RHBDF2	453	TCL1A
22	ANXA13	94	CX3CR1	166	GCHFR	238	LOXL3	310	OCLN	382	RHEB	454	TCL1B
23	APBA3	95	CYTH1	167	GFI1	239	LPAR2	311	ONECUT1	383	RIPK2	455	TDRD1
24	APH1A	96	DACH2	168	GJA1	240	LPAR4	312	OPA1	384	RIPK3	456	TFR2
25	APPL1	97	DACT3	169	GLI1	241	LPAR5	313	OTOR	385	RIT1	457	TGFB1I1
26	ARAF	98	DAXX	170	GLIS1	242	LPL	314	PADI2	386	RIT2	458	TIAM1
27	ARHGEF12	99	DDB2	171	GPR101	243	LPP	315	PAK1	387	RND3	459	TICAM2
28	ASCL2	100	DDIT3	172	GPR12	244	LRMP	316	PAK3	388	RNF14	460	TINAG
29	ATAD2	101	DDX17	173	GPR183	245	LRP1B	317	PALLD	389	RNF8	461	TLE1
30	ATF1	102	DDX5	174	GPR22	246	LRP5	318	PANX2	390	RORA	462	TLR5
31	ATF4	103	DDX58	175	GPR26	247	LRRK2	319	PARD3	391	RPS6KA6	463	TM4SF1
32	ATG5	104	DECR1	176	GPR34	248	MAD1L1	320	PAWR	392	RUNX1	464	TMEM59
33	ATN1	105	DGKB	177	GPR37L1	249	MAF	321	PAX5	393	RXRA	465	TMPRSS9
34	AURKB	106	DGKD	178	GPRC5A	250	MAFF	322	PAX6	394	RXRB	466	TNKS
35	AXIN2	107	DGKI	179	GPRC5B	251	MAFG	323	PCDH19	395	RXRG	467	TNS4
36	BACH1	108	DGKZ	180	GSK3A	252	MAFK	324	PCDHA1	396	SACS	468	TRAF1
37	BCR	109	DISP2	181	GUCY2C	253	MAOA	325	PDZK1	397	SALL1	469	TRAF5
38	BRCA1	110	DMPK	182	GZMK	254	MAP2	326	PEA15	398	SALL4	470	TRAF6
39	BRIX1	111	DNAH17	183	HAX1	255	MAP2K3	327	PHOX2B	399	SAMHD1	471	TRIM28
40	BTN3A2	112	DNMT1	184	HES1	256	MAP2K4	328	PIBF1	400	SATB1	472	TRIM5
41	BTRC	113	DOCK2	185	HEXIM1	257	MAP3K10	329	PIK3CB	401	SCAMP3	473	TSC2
42	C12orf5	114	DOCK3	186	HHEX	258	MAP3K11	330	PIK3CD	402	SCGB3A2	474	TSPAN2
43	C1QTNF3	115	DOK3	187	HIF1AN	259	MAP3K14	331	PIK3R2	403	SGCA	475	TSPAN9
44	C3AR1	116	DPPA2	188	HIST3H3	260	MAP3K3	332	PIK3R4	404	SGCD	476	TSPO
45	C5AR1	117	DRD1	189	HMGA1	261	MAP3K7IP1	333	PIK3R5	405	SHB	477	TWIST2
46	C6orf190	118	DRD2	190	HMGA2	262	MAP4K5	334	PINK1	406	SIGMAR1	478	UCP1
47	CARD9	119	DUSP1	191	HNF1B	263	MAPK11	335	PIWIL4	407	SIN3A	479	UCP2
48	CAV3	120	DVL1	192	HNF4G	264	MAPK7	336	PKD1	408	SLC12A2	480	VISA
49	CBLN4	121	DVL3	193	HOXB13	265	MATN1	337	PLA2G16	409	SLC16A1	481	VSIG10L
50	CCNA2	122	E2F1	194	HR	266	MATN4	338	PLCB1	410	SLC17A7	482	VWA1
51	CCND3	123	E2F2	195	HS6ST2	267	MBD3	339	PLCB3	411	SLC18A2	483	WASF1
52	CCNE2	124	EBF1	196	HSD11B2	268	MCPH1	340	PLCD3	412	SLC1A3	484	WASF3
53	CCR10	125	EBF2	197	HSF2	269	MDC1	341	PLCG2	413	SLC22A1	485	WDR5
54	CCRL1	126	EBF3	198	HSF4	270	MEGF9	342	PLD1	414	SLC22A2	486	WNT10A
55	CD1B	127	EGFL7	199	ICA1	271	MELK	343	PLD2	415	SLC27A1	487	WNT6
56	CD1C	128	EGLN1	200	ID2	272	MEN1	344	PLEKHA1	416	SLC27A2	488	XBP1
57	CD1D	129	EGLN2	201	IFITM3	273	MFN1	345	PLSCR1	417	SLC27A5	489	XG
58	CD1E	130	EGLN3	202	IFNA17	274	MGMT	346	POU3F2	418	SLC6A3	490	ZBTB17
59	CDC73	131	EIF2AK3	203	IFNA6	275	MITF	347	PPARA	419	SLC7A5	491	ZBTB7A
60	CDK8	132	EIF2AK4	204	IFNGR2	276	MLKL	348	PPARD	420	SLC8A1	492	ZEB1
61	CDKN2AIP	133	EIF4B	205	IGDCC3	277	MLX	349	PPARGC1A	421	SMAGP	493	ZEB2
62	CDKN2B	134	EIF4G1	206	IKBKB	278	MRC2	350	PPM2C	422	SMARCA5	494	ZFP90
63	CDKN2C	135	EMCN	207	IKZF1	279	MSH2	351	PPP1R1B	423	SMO	495	ZG16B
64	CDX2	136	ENAH	208	IKZF3	280	MSX2	352	PRDM16	424	SNAPIN	496	ZMIZ1
65	CEBPE	137	ENPP3	209	IL17RE	281	MTF2	353	PREX1	425	SOCS4	497	ZNF366
66	CELSR2	138	EPAS1	210	IL4I1	282	MUC19	354	PRF1	426	SOS2	498	ZNF71
67	CFTR	139	EPS8	211	IRAK1	283	MX1	355	PRKAA2	427	SOX18	499	ZSCAN10
68	CHD1L	140	ESRRG	212	IRAK2	284	MXI1	356	PRKAB2	428	SOX5	500	ZSCAN21
69	CHRM3	141	ETV1	213	IRF2BP1	285	MYOCD	357	PROKR1	429	SOX6		
70	CHRM5	142	ETV5	214	ITGA8	286	MYOD1	358	PROM2	430	SP3		
71	CHUK	143	ETV6	215	ITGA9	287	NANOS2	359	PRSS22	431	SP7		
72	CIDEC	144	EZH2	216	ITGB1BP1	288	NCOA3	360	PSENEN	432	SPIB		

VII. Interpretation of Results:

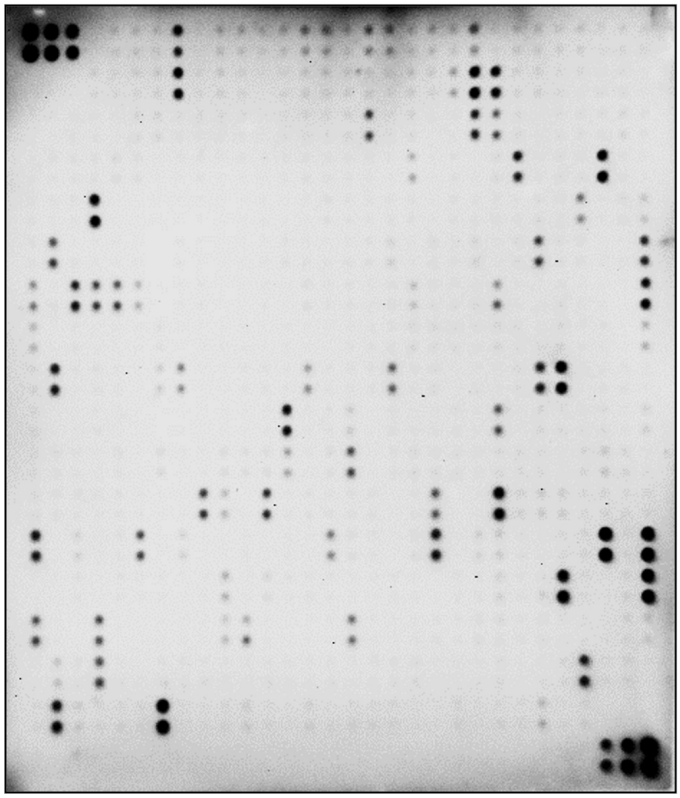
A. Explanation of Controls Spots

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.

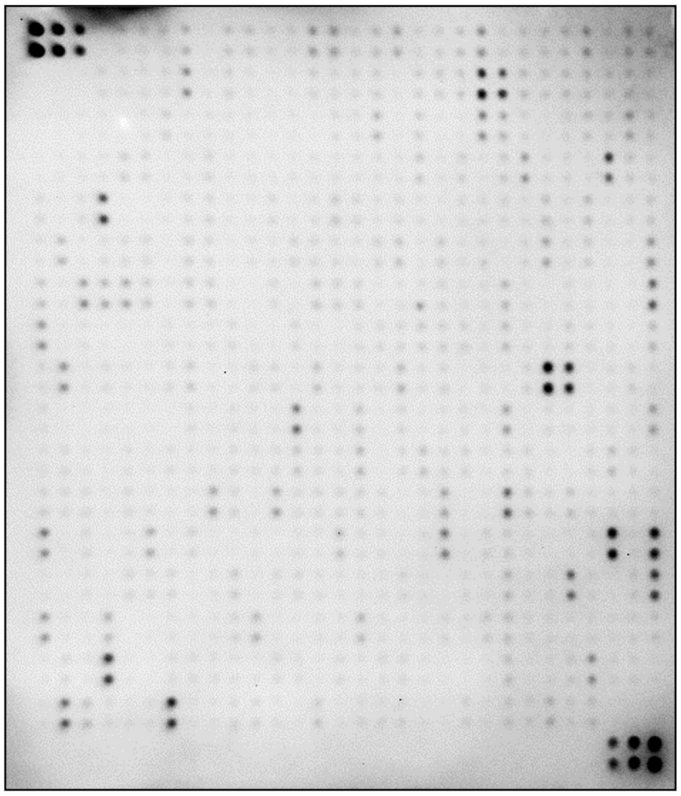
B. Typical Results

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

Human Serum



Human Plasma



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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