RayBio® Label-Based (L-Series) Human L7 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-7-2 (2 Sample Kit)
AAH-BLM-7-4 (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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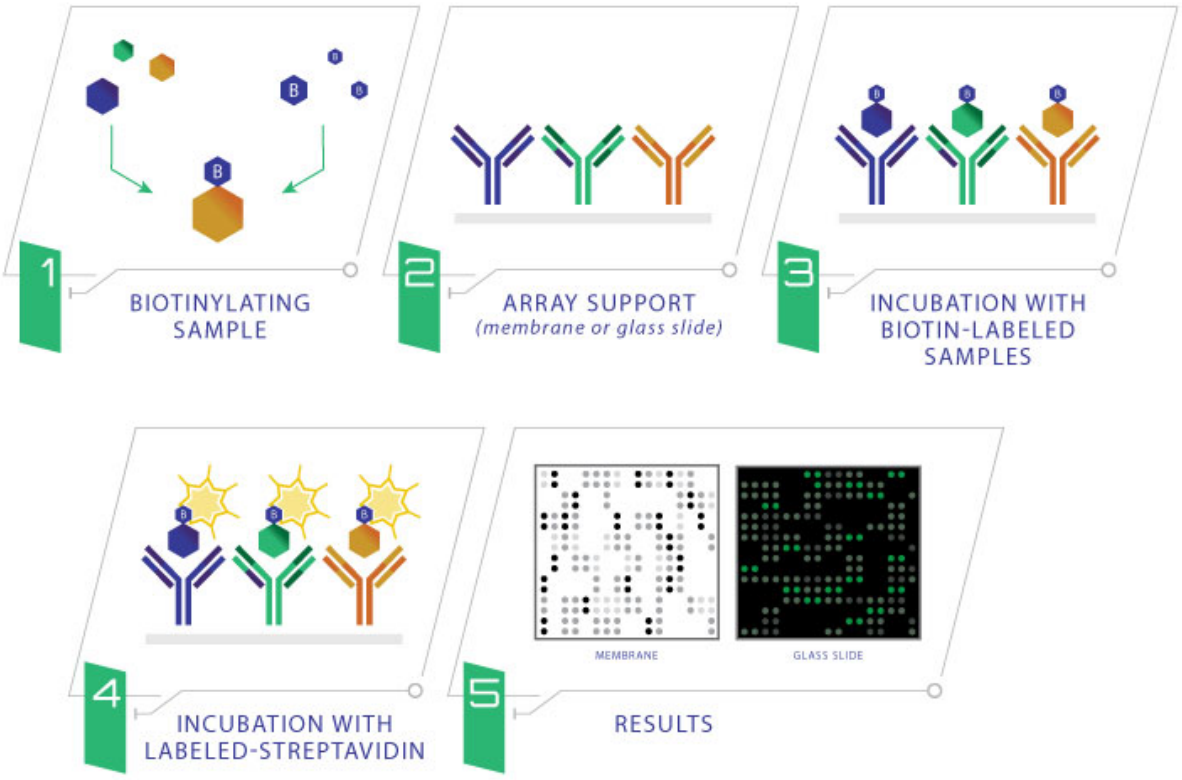
I. Introduction

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).

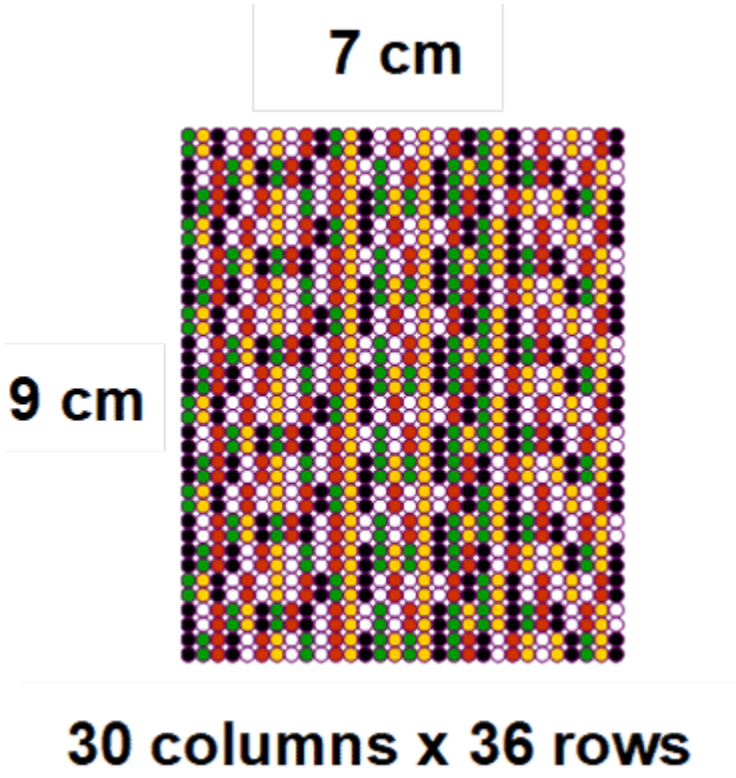
B. Handling the Array Membranes

- 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.

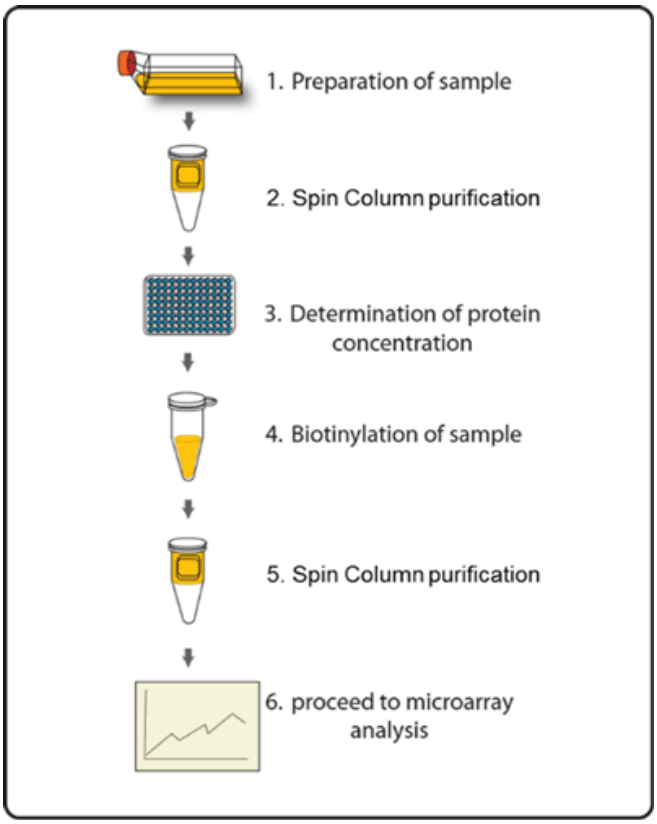
D. Layout of Array Membrane



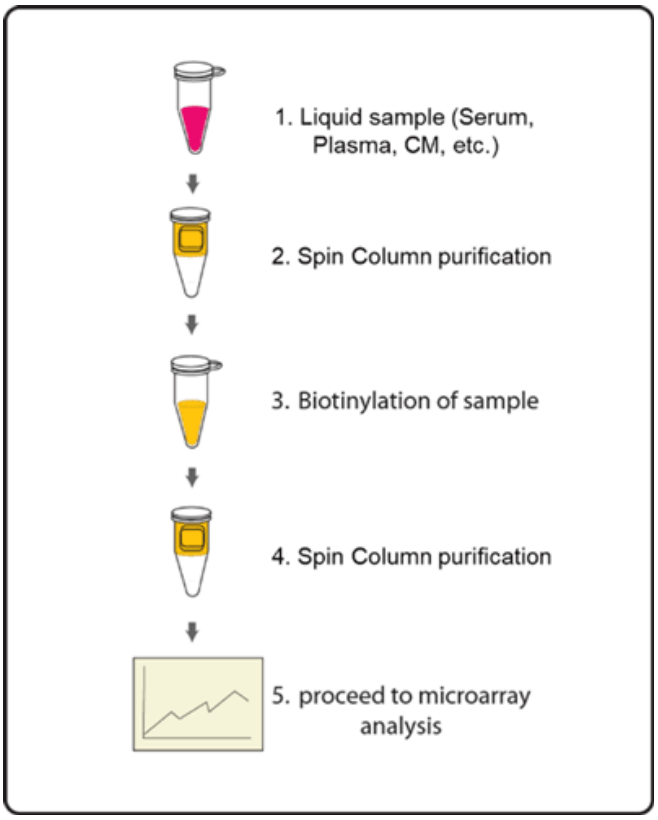
IV. Protocol

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
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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	AAMDC	73	CBX1	145	DDB1	217	FN3KRP	289	MSH4	361	PPID	433	SRRM4
2	ABCC8	74	CBX3	146	DDRGK1	218	FTSJ3	290	MTCOX3	362	PPP1R13B	434	SS18
3	ABCF2	75	CBX8	147	DDX19A	219	FXYD2	291	MTFR2	363	PPP1R3C	435	SSB
4	ABHD11	76	CCDC114	148	DDX19B	220	FXYD3	292	MUC15	364	PPP3CB	436	SSPN
5	ABHD16A	77	CCDC124	149	DDX24	221	G6PC	293	MYBPH	365	PRPH	437	SSX2
6	ACOT9	78	CCDC40	150	DDX39A	222	GADD45B	294	NAB2	366	PSMC2	438	ST8SIA6
7	ACSF2	79	CCDC47	151	DDX47	223	GALK2	295	NCF1	367	PSMC4	439	STARD10
8	ACTL6A	80	CCDC6	152	DDX50	224	GATAD2A	296	NCOA4	368	PSMC5	440	STRADA
9	ACTL6B	81	CCIN	153	DDX53	225	GATAD2B	297	NDN	369	PSMC6	441	SUPT3H
10	ACY3	82	CCM2L	154	DDX56	226	GBAS	298	NDUFA10	370	PSMD13	442	SUPT7L
11	ADAMTSL4	83	CCND2	155	DENR	227	GCA	299	NDUFA13	371	PSMD14	443	SZRD1
12	ADCY5	84	CCNF	156	DERL1	228	GFPT2	300	NDUFA2	372	PSMD2	444	TAF4B
13	ADCY7	85	CCNT1	157	DGCR8	229	GHITM	301	NDUFA3	373	PSMD6	445	TAF7L
14	ADD1	86	CCT5	158	DIMT1L	230	GIMAP1	302	NDUFA5	374	PSMD7	446	TANGO2
15	ADRM1	87	CD2BP2	159	DLEC1	231	GIMAP6	303	NDUFB5	375	PYGM	447	TC2N
16	AFAP1L2	88	CDC23	160	DLGAP4	232	GJA8	304	NDUFB7	376	R3HDM2	448	TET1
17	AFF4	89	CDC42EP4	161	DNAH8	233	GJA9	305	NDUFB8	377	RAB18	449	TFAM
18	AGO3	90	CDCA5	162	DNAJB4	234	GJB6	306	NDUFB9	378	RAB38	450	THAP12
19	AHCTF1	91	CDCA8	163	DNAJC8	235	GLMN	307	NDUFC1	379	RABEP1	451	TLX2
20	AHNAK2	92	CDK9	164	DNAL1	236	GNA15	308	NDUFS2	380	RAC3	452	TMEM106C
21	AIPL1	93	CEACAM16	165	DNTTIP2	237	GNB5	309	NDUFS3	381	RAD9B	453	TMEM8B
22	ANKEF1	94	CEACAM21	166	DPF2	238	GNGT2	310	NDUFS6	382	RASL10A	454	TMPRSS2
23	ANKRD30B	95	CENPI	167	DPH5	239	GNL1	311	NDUFV2	383	RASSF1	455	TNN
24	AP2A2	96	CEP55	168	DR1	240	GOLGA1	312	NEMF	384	RASSF5	456	TOMM40L
25	AP2B1	97	CGAS	169	DRAP1	241	GORASP2	313	NOG	385	RBL1	457	TPM2
26	APBB1	98	CGGBP1	170	DROSHA	242	GPN1	314	NR5A2	386	RBM6	458	TRABD
27	APEH	99	CHCHD5	171	DUSP26	243	GRPEL1	315	NRSN2	387	RCAN3	459	TRIB3
28	APOBR	100	CHD4	172	ECI2	244	GSTA3	316	NUDCD1	388	RCVRN	460	TRIM13
29	ARHGAP12	101	CHD5	173	EIF2D	245	GSTM3	317	NUDT13	389	REM1	461	TRIM36
30	ARHGAP4	102	CHML	174	EIF3M	246	GTF2A1L	318	OARD1	390	RFPL2	462	TRIM42
31	ARHGEF1	103	CHMP2A	175	EIF4ENIF1	247	GYS2	319	OAZ2	391	RHOBTB2	463	TRIM43
32	ARHGEF6	104	CIP2A	176	EIF4H	248	H1F0	320	OCIAD1	392	RIPK4	464	TRIM55
33	ARIH1	105	CISD2	177	ELAC2	249	HAP1	321	OCIAD2	393	RNF115	465	TRMO
34	ARL1	106	CLASP1	178	EN1	250	HAUS1	322	OGT	394	RSPH14	466	TRMT10C
35	ARL3	107	CLCC1	179	ENDOD1	251	HAUS7	323	PACRG	395	RTN3	467	TRMU
36	ARL6	108	CLIC3	180	ENOX2	252	HDAC11	324	PAQR6	396	RTP4	468	TROVE2
37	ARMC1	109	CLPP	181	EPB41L3	253	HKDC1	325	PARP12	397	SARG	469	TSPAN17
38	ASB11	110	CLYBL	182	EPB42	254	HLA-E	326	PBX1	398	SCGB2A1	470	TTC16
39	ASB12	111	CMC1	183	EPS8L2	255	HOPX	327	PBXIP1	399	SCNN1B	471	TTC23
40	ASB6	112	CNPY3	184	EPS8L3	256	HS3ST6	328	PCDH12	400	SEC14L4	472	TTC6
41	ASMTL	113	CNTNAP4	185	ERCC6L	257	HYPK	329	PCDHA10	401	SEL1L3	473	TUBA1B
42	ATG13	114	COA6	186	ESPN	258	IFT57	330	PCDHAC1	402	SEPTIN12	474	TUBB4A
43	ATG2A	115	COBLL1	187	ETFB	259	JCAD	331	PCDHAC2	403	SHC2	475	TUBE1
44	ATP13A3	116	COL5A3	188	EURL	260	JPH3	332	PCDHGA4	404	SIGLEC12	476	UBA52
45	ATP1B2	117	COL8A1	189	EXOC5	261	KCNQ1	333	PCDHGB1	405	SLC12A3	477	UBAP1
46	ATP1B3	118	COLCA1	190	EXPH5	262	KCTD9	334	PCDHGB3	406	SLC15A2	478	UBE2J1
47	ATP1B4	119	COMMD10	191	F2RL3	263	KIF20B	335	PCDHGC4	407	SLC16A6	479	UGT1A6
48	ATP2A1	120	COPG1	192	FADS3	264	KIF5C	336	PCED1A	408	SLC1A5	480	UQCRC1
49	ATP6V0D1	121	COPS2	193	FAIM	265	KIF9	337	PCYT1A	409	SLC20A1	481	UQCRC2
50	ATP6V0E2	122	CORO1B	194	FAM107A	266	KLC3	338	PDHA1	410	SLC23A2	482	USP32
51	ATPAF2	123	COX5A	195	FAM111B	267	KLC4	339	PDZRN4	411	SLC26A1	483	VAV2
52	ATXN10	124	COX6B1	196	FAM129B	268	KNSTRN	340	PELI3	412	SLC2A8	484	WBP1L
53	ATXN2	125	CREB3L2	197	FAM13C	269	LACTB	341	PFKFB3	413	SLC2A9	485	WDR23
54	ATXN7L2	126	CRISPLD1	198	FAM175B	270	LCN8	342	PFTK1	414	SLC35E3	486	WDR34
55	BCAS2	127	CRISPLD2	199	FAM181B	271	LDOC1	343	PHACTR1	415	SLC36A4	487	WDR35
56	BCAS3	128	CRMP1	200	FAM217B	272	LETMD1	344	PHF11	416	SLC38A2	488	WDTC1
57	BRME1	129	CRTAP	201	FAM45BP	273	LYRM1	345	PIK3C3	417	SLC3A1	489	WHSC1
58	BRWD1	130	CRYM	202	FAM53C	274	LZTS2	346	PIP5K1B	418	SLC43A1	490	WNT1
59	BTF3L4	131	CTDSPL	203	FAM96B	275	MAGEA10	347	PKD2	419	SLC44A1	491	WNT2
60	BZW2	132	CTNNA3	204	FAM98B	276	MAGEB10	348	PKD2L2	420	SLC44A3	492	WNT5B
61	C12orf57	133	CTR9	205	FASTKD3	277	MED19	349	PLA2G4F	421	SLC4A1AP	493	WRB
62	C1QTNF6	134	CWF19L1	206	FBXL16	278	MGST1	350	PLCD1	422	SLC7A2	494	XP32
63	C20orf144	135	CYP1B1	207	FBXL2	279	MGST2	351	PLCL1	423	SLC7A7	495	ZBED6CL
64	C5orf22	136	CYP24A1	208	FBXO10	280	MIA3	352	PLEKHA4	424	SLC9A1	496	ZDHHC19
65	C7orf25	137	CYP2A13	209	FBXO16	281	MIS18A	353	PLEKHA8	425	SLCO2B1	497	ZGPAT
66	C9orf78	138	CYP2E1	210	FBXO33	282	MKL1	354	PMEL	426	SLCO3A1	498	ZNF14
67	CAMK2B	139	DAP3	211	FBXO39	283	MLF2	355	PNOC	427	SMG8	499	ZPBP2
68	CAND1	140	DAPK2	212	FBXO42	284	MLLT3	356	POLB	428	SNCAIP	500	ZSWIM3
69	CAPN6	141	DBNL	213	FDX1	285	MORC1	357	POLR2B	429	SNRPD1		
70	CAPN9	142	DBR1	214	FDXR	286	MROH8	358	POLR2C	430	SNTB1		
71	CAPRIN1	143	DCTN3	215	FGD6	287	MRPL47	359	POLR2D	431	SNW1		
72	CAPRIN2	144	DCTN6	216	FLVCR1	288	MRPL58	360	PPHLN1	432	SPECC1L		

VII. Interpretation of Results:

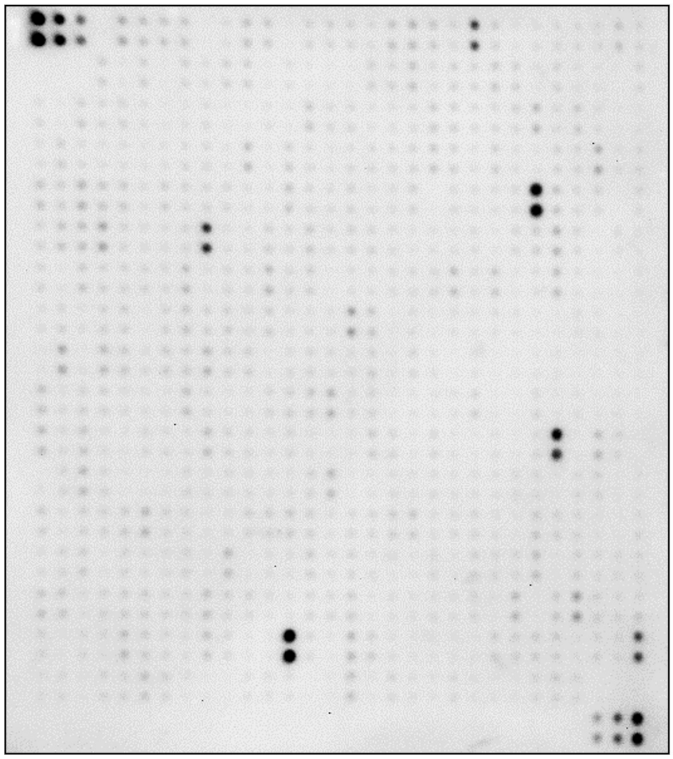
A. Explanation of Controls Spots

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



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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