RayBio® Label-Based (L-Series) Human L4 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-4-2 (2 Sample Kit)
AAH-BLM-4-4 (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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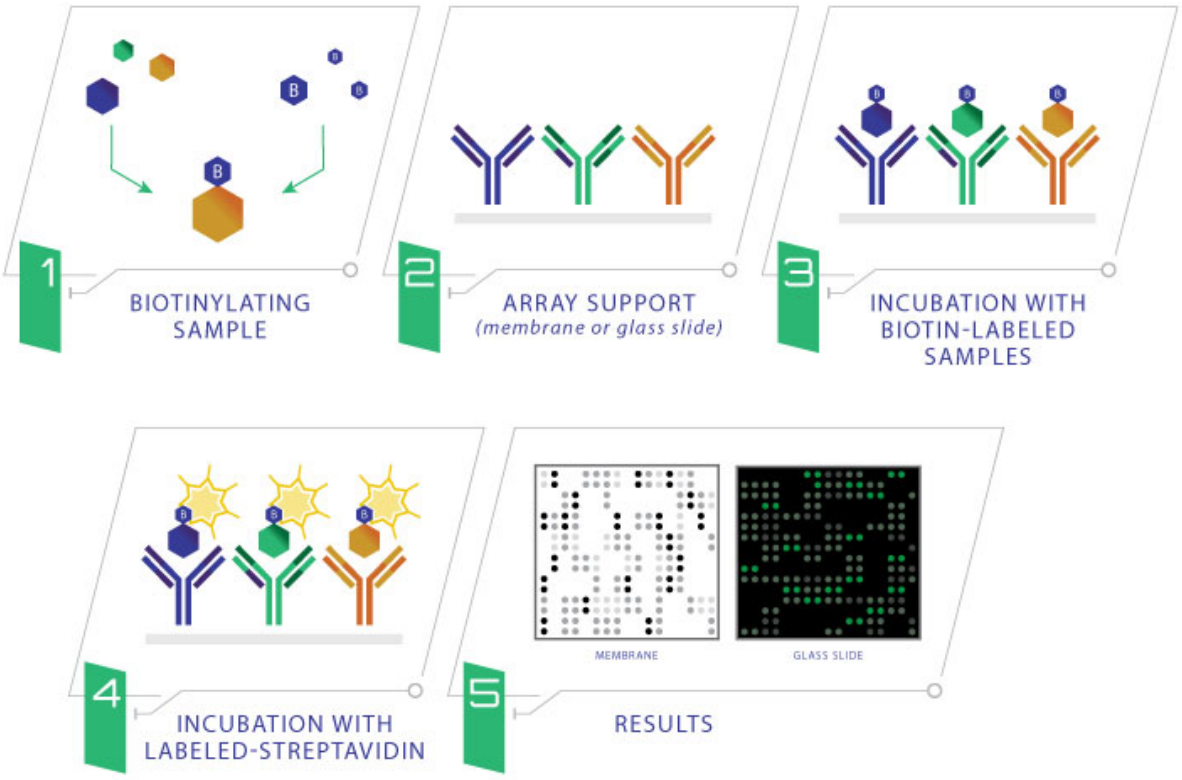
TABLE OF CONTENTS

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).

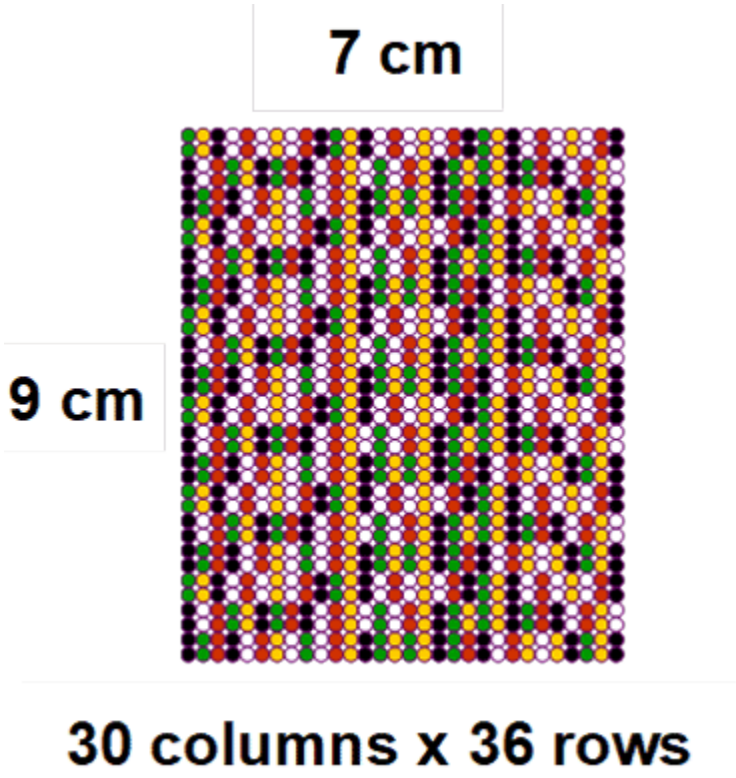
B. Handling the Array Membranes

- 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.

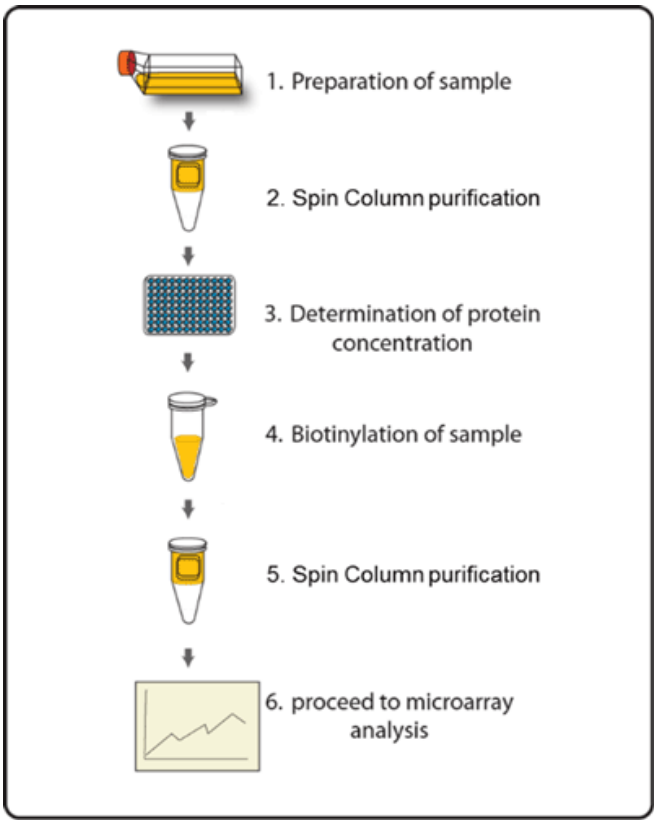
D. Layout of Array Membrane



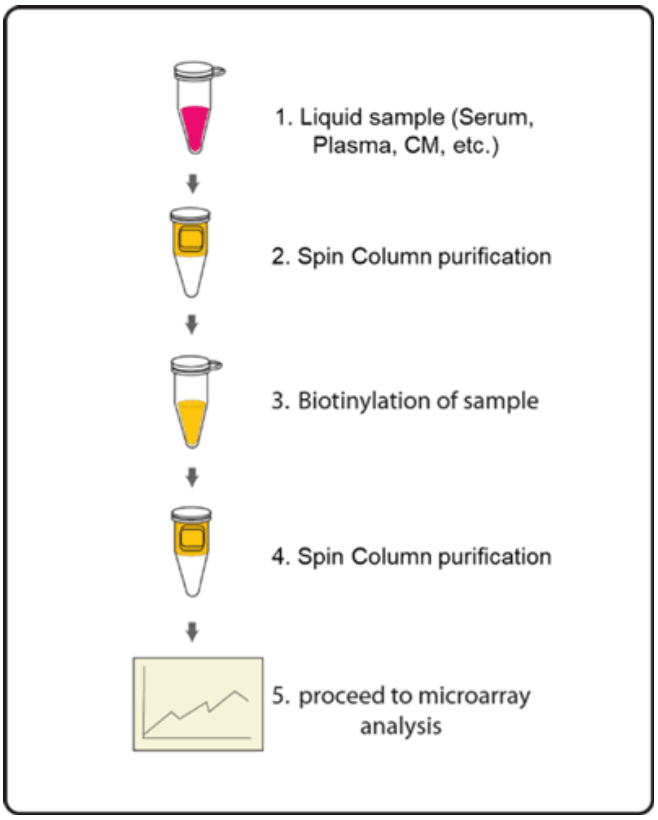
IV. Protocol

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	HEXA	73	LIMS1	145	Nectin-1	217	Peroxiredoxin 3	289	PTK 7	361	Serpin A7	433	Thymosin b10
2	HTRA1	74	LMAN2	146	Nectin-3	218	Peroxiredoxin 5	290	PTMA	362	Serpin B3	434	Titin
3	Agrin	75	ACP1	147	NEDD8	219	PF4V1	291	PTP gamma	363	Serpin B6	435	TLS
4	IBP160	76	LOK	148	Neogenin	220	PGAM1	292	PTP kappa	364	Serpin B8	436	TMEM223
5	IDH1	77	LOX	149	Nesprin2	221	PGAM2	293	PTP mu	365	Serpin F2	437	TOB2
6	IDH3A	78	LOXL1	150	Neurabin 1	222	PGD	294	PTPRS	366	Serpin A10	438	TOP2B
7	IFRD1	79	LRP 4	151	Neural Cadherin	223	PHGDH	295	PTPRZ	367	SERPINB1	439	TPM4
8	IGF2BP2	80	LTA4H	152	PAM	224	PGK-1	296	PYGL	368	SerpinB4	440	TPP1
9	ITGB5	81	LTBP4	153	Neurogranin	225	PGLS-C-t	297	PZP	369	SerpinE2	441	TALDO1
10	IGSF4B	82	Lubricin	154	Neuropeptide B	226	PGM1	298	QDPR	370	SerRS	442	TALDO
11	Ihh	83	LUZP1	155	Neuropilin-1	227	PGRPL	299	QPRT	371	SET	443	Transthyretin
12	ILK	84	LYPA1	156	Neurotrimin	228	PHAP1	300	Quiescin Q6	372	SEZ6L2	444	TRAP1
13	Inhibin beta	85	Lysozyme	157	NF-M	229	PSAT1	301	Rab7a	373	SF20	445	TRAP220
14	ITGB1	86	MAGI2	158	Nidogen-2	230	PIK3C2B	302	Ran	374	SH3BGRL	446	TRF 2
15	ITGB6	87	MAGP-2	159	NIT2	231	pIgR	303	RanGAP1	375	SH3BGRL3	447	TPIS
16	ITGA6	88	MAN1	160	NME3	232	PIK3IP1	304	RAP1AB	376	SHANK1	448	Tropomyosin 3
17	IQGAP1	89	MANF	161	nNOS	233	PIN	305	Rbm15	377	SHC1	449	Twist-1
18	IQGAP2	90	Mannosidase II	162	Noelin	234	PISD	306	RCL	378	SHIP	450	TRPS1
19	IRE1	91	MAP1A	163	Non-muscle Actin	235	PKLR	307	Reg1A	379	SHMT1	451	Trypsinogen-2
20	IRS2	92	MAPRE1	164	Myosin IIA	236	PLA2G1B	308	Reg3A	380	SHP-1	452	Trypsin Pan
21	ISOC2	93	MARCKS	165	Notch-2	237	Plakophilin 1	309	RHOC	381	Siglec-1	453	WRS
22	ITGB4BP	94	MASP3	166	Notch-2 ICD	238	Plastin L	310	RhoGDI	382	SIGLEC14	454	TSR2
23	ITIH1	95	MBD2	167	NPAS3	239	PLC-gamma 1	311	RNASE1	383	SIM2	455	TUBA6
24	ITIH2	96	MBP	168	NPM1	240	Pleckstrin	312	RNH1	384	SIRP beta 1	456	TWF2
25	ITIH3	97	MCAM	169	NQO2	241	Plectin	313	RNASET2	385	Six3	457	TXNDC4
26	ITIH4 a	98	Mcl-1	170	NT5C3	242	Plexin B1	314	RKIP	386	SLC38A10	458	TXNDC5
27	JAM-A	99	MCM	171	NUCB1	243	Plexin B2	315	POLR2A	387	SLITRK1	459	TXNRD2
28	JARID2	100	MCM5	172	NUP98	244	PLOD1	316	RNASE4	388	SLURP1	460	UBE2D3
29	KPNB1	101	MCMP2	173	OBCAM	245	PLOD2	317	RNASE6	389	SMA	461	Ube2L3
30	Keratin 36	102	MDH1	174	OIT3	246	PLS3	318	RPL10	390	SMC4	462	UBE2N
31	Keratin 38	103	MDH2	175	Olfactomedin-2	247	Plxdc2	319	RPL10A	391	SMPD4	463	Ubiquitin
32	KHSRP	104	ME1	176	OTC	248	PNP	320	RPL11	392	SOD1	464	UCH-L1
33	KIAA0319L	105	MEP1A	177	Orosomucoid 2	249	POR	321	RPL12	393	SOD2	465	UFM 1
34	KIAA1468	106	Metallothionein	178	ORP150	250	PPCS	322	RPL14	394	SOD-3	466	UGGT
35	KIAA1967	107	Metavinculin	179	OSBP1	251	PPOX	323	RPL17	395	SOD4	467	UNC13D
36	KIF5B	108	MFAP4	180	OSCAR	252	PPP2R1B	324	RPL22	396	Somatostatin	468	UNC45A
37	Kilon	109	MFI2	181	OSM R beta	253	PPP2R4	325	RPL5	397	SORD	469	UNC5H4
38	KLK-B1	110	mGLUR5	182	Osteoadherin	254	PRCP	326	RPL7A	398	SorLA	470	UPB1
39	KMD4B	111	MGP	183	OXT	255	PRDM13	327	RPLP0	399	SOX4	471	UQCRB
40	KMT2B	112	Mimecan	184	p16 ARC	256	PRDX 1	328	RPS10	400	SP-D	472	UQCRH
41	KRT31	113	MINPP1	185	P20Sb3	257	PRELP	329	RPS11	401	Spectrin beta-5	473	URB
42	KRT72	114	MLCK	186	p23	258	PREP	330	RPS12	402	SPEN	474	URB2
43	Krt73	115	MMR	187	p39	259	PRG2	331	RPS19	403	SPINK7	475	UROC1
44	KRT82	116	MMRN1	188	P4HB	260	PRNP	332	RPS2	404	SPTBN1	476	UROD
45	KRT85	117	MN1	189	p73	261	Profilin 1	333	RPS20	405	Src	477	URP2
46	KRTDAP	118	Moesin	190	PA2G4	262	Properdin	334	RPS23	406	SREC-II	478	USP14
47	KRTHA3B	119	MP1	191	PABP	263	Prosaposin	335	RPS25	407	STAT3	479	USP5
48	KSR1	120	MPCA	192	PACS1	264	PTGDS	336	RPS28	408	Stathmin 1	480	Uteroglobin
49	LAD	121	MPO	193	PARVB	265	PSMB6	337	RPS3	409	SCP2	481	Utrophin
50	LAF4	122	MRP 1	194	PCBP1	266	PSMA3	338	RPS5	410	STI1	482	VAP-1
51	LAIR1	123	MSH6	195	PCBP2	267	PSMA5	339	RREB1	411	STOM	483	VAP-A
52	LAM b1	124	mTOR	196	PCCA	268	PSMB7	340	RSU1	412	SUCLG1	484	VCP
53	LAMA	125	MUCDHL	197	PCDH7	269	PSMD5	341	S100A1	413	SUMO3	485	VDAC1
54	LMNA	126	Multimerin 2	198	PCDX8	270	PSMB1	342	S100A11	414	Symplekin	486	Versican
55	LMNB1	127	MyBPC3	199	PCK2	271	PSMA6	343	S100A7	415	SynCAM	487	Vimentin B
56	LMNB2	128	MYH2	200	PCMT1	272	PSB2	344	S100A9	416	Syntaxin 7	488	VNN1
57	LAMA2	129	MYH6	201	PCNA	273	PSB4	345	S100P	417	TAB182	489	VSIG4
58	LAMB2	130	MYH7	202	PCPE-1	274	Protein C	346	TIM-4	418	TAGLN2	490	WDR1
59	LAMC1	131	MYHC	203	PCSK9	275	Protein Z	347	SAA4a	419	Talin1	491	WISP2
60	LAMP	132	MYL12B	204	PCYOX1	276	Prouroguanylin	348	aAmylase	420	Talin1&2	492	WNK2
61	LAMP1	133	MYL3	205	PDE1B	277	PRSS23	349	SAMSN1	421	TAX1BP3	493	YB1
62	LAMP2	134	MYO5A	206	PDIA6	278	PRSS3	350	SBP-1	422	TBCA	494	YY1
63	LAP3	135	Myoferlin	207	PDLIM1	279	PRTN3	351	SBSN	423	TCEB2	495	ZBTB4
64	LASP1	136	Myosin 18B	208	PDLIM5	280	PSMA1	352	SDF4	424	Tcf20	496	ZC3H4-N-t
65	LTBP2	137	Myotrophin	209	PDZD2	281	PSMA2	353	SDNSF	425	TCN1	497	ZC3H8
66	LCAT	138	NABC1	210	PEBP4	282	PSMA4	354	SDPR	426	TCP1 eta	498	ZDHHC18
67	LCMT2	139	NAGLU	211	PEPD	283	PSMA7	355	SCG5	427	Tenascin C	499	ZNF671
68	LDHA	140	NAP1L1	212	PER1	284	PSMB5	356	Semaphorin 6B	428	Tenascin X	500	Zyxin
69	LDHB	141	NAPRT1	213	perilipin 3	285	PSMC3	357	Semaphorin 7A	429	TFF2		
70	LEDGF	142	NASP	214	Perilipin-1	286	PSMD1	358	SEMG1	430	TGM3		
71	SPINK5	143	NCAM2	215	Periostin	287	PSMD9	359	SEMG2	431	Thioredoxin-1		
72	LILRA3	144	Nebulin	216	Peroxiredoxin 2	288	PTEN	360	Serpin A11	432	THOP1		

VII. Interpretation of Results:

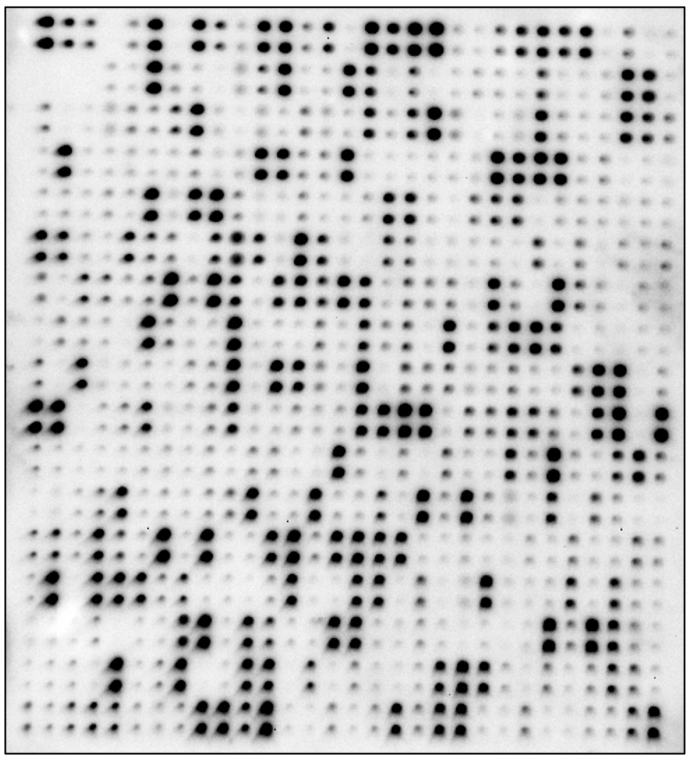
A. Explanation of Controls Spots

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.

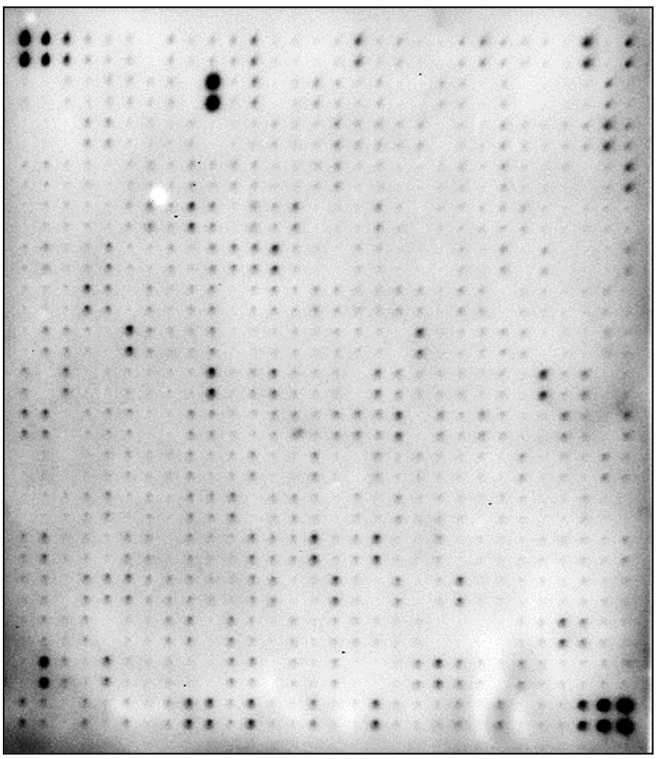
B. Typical Results

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

Human Serum



Sample A



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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