RayBio® Label-Based (L-Series) Rat L4 Array, Membrane

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAR-BLM-4-2 (2 Sample Kit)
AAR-BLM-4-4 (4 Sample Kit)

Please read manual carefully before starting experiment

Your Provider of Excellent Protein Array Systems and Services

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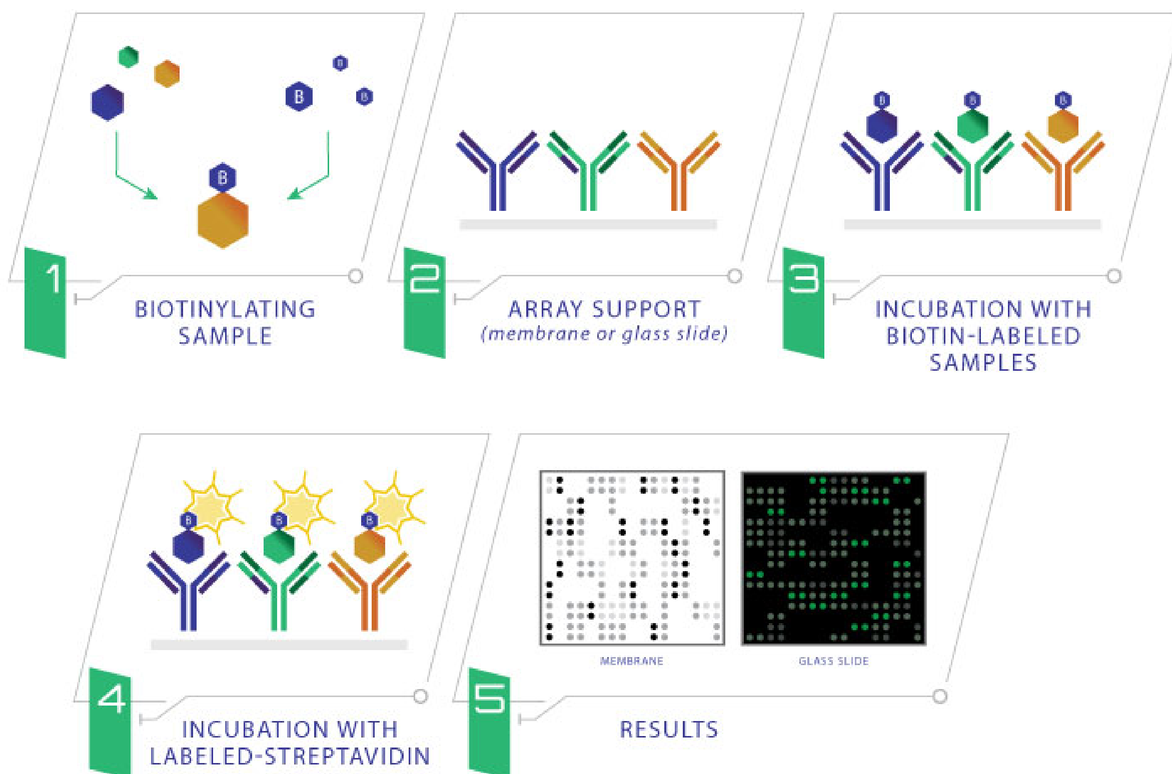
I. Introduction

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).

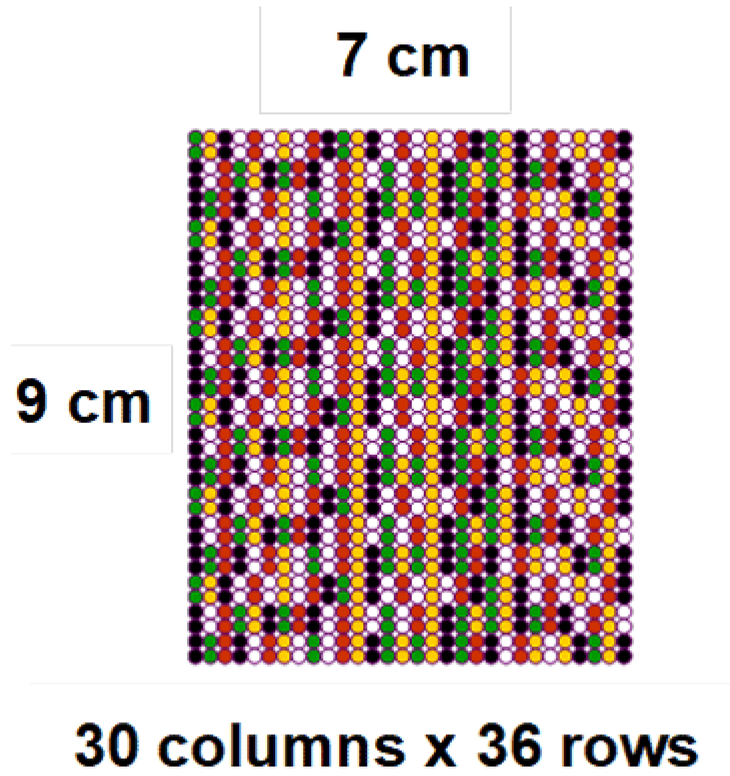
B. Handling the Array Membranes

- 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.

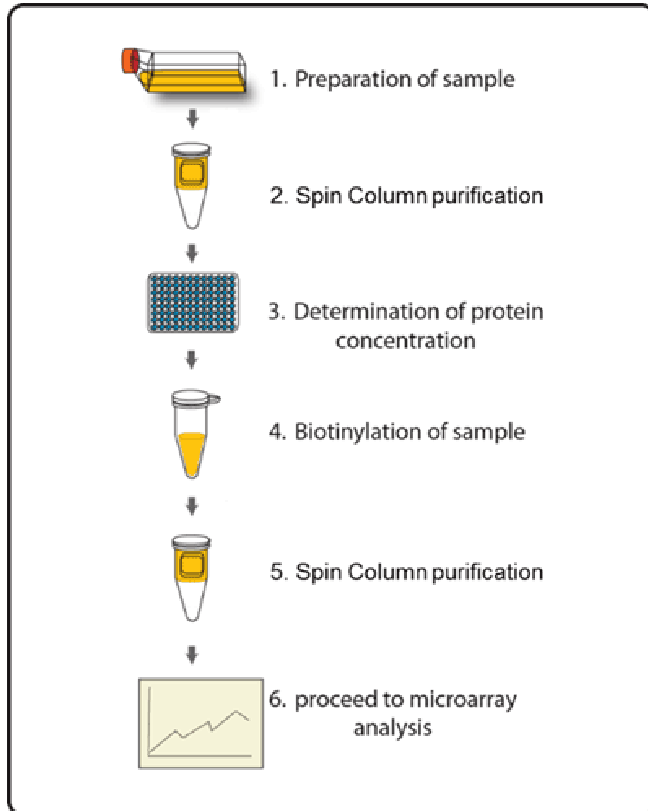
D. Layout of Array Membrane



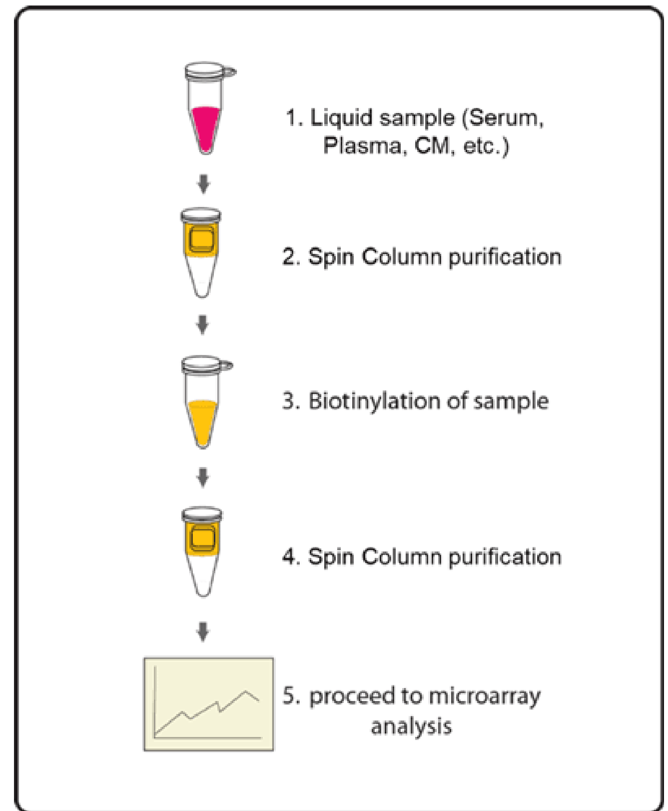
IV. Protocol

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
1	POS1	POS2	POS3	Blank	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
2	POS1	POS2	POS3	Blank	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
3	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
4	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
5	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
6	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83
7	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113
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33	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	Blank	Blank	Blank
34	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	Blank	Blank	Blank
35	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS3	POS2	POS1
36	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	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	14-3-3 beta	73	CD40	145	FCGR3A	217	IMPAD1	289	NDFIP1	361	PNUTS	433	SHIP
2	14-3-3 gamma	74	CD44	146	FCGRT	218	IMPDH2	290	Nectin-3	362	PP2A	434	SHP-2
3	A1BG	75	CD51	147	Fen 1	219	Inhibin beta	291	Nesfatin-1	363	PPM1B	435	SIGNR3
4	A1M	76	CD59	148	Filamin A	220	iNOS	292	Nesprin2	364	PPM1L	436	Six3
5	aAmylase	77	CDC25A	149	FKBP38	221	Intelectin-1	293	Neurogenin-2	365	PPP1R9B	437	SMAGP
6	ACE2	78	CDC25C	150	FoxA2	222	IRE1	294	Neuroglycan C	366	PRCP	438	SMOC-1
7	ACLP	79	CDK1	151	FoxP3	223	IRS1	295	NGFR	367	PRDX5	439	SMURF2
8	ACTN2	80	CDK2	152	FPRP	224	IRS2	296	Nicalin	368	PRG2	440	SNAP25
9	ADAM17	81	CEACAM1	153	FSTL4	225	ITGA8	297	Ninjurin-2	369	PRNP	441	SOD1
10	ADAM9	82	CELF1	154	FUCA1	226	ITGB4BP	298	NIPP1	370	Prohibitin	442	SOD2
11	ADNP	83	CES3	155	Fyn	227	ITGB5	299	NKX2.2	371	Prss21	443	SOD-3
12	ADRB2	84	CHORDC1	156	G3BP	228	ITGB6	300	NLRP10	372	PSD-95	444	SPOCK2
13	AFP	85	CKBB	157	G6PD	229	ITPR3	301	NPC1	373	PTEN	445	SQSTM1
14	AGT	86	CLEC1B	158	GABAB R1	230	JAB1	302	NR3C1	374	PTGDS	446	SR-AI
15	Akt1	87	CLEC5A	159	GABAB R2	231	Jak1	303	NrCAM	375	PTGES3	447	ST3GAL2
16	ALDH2	88	COL1A1	160	GABRA1	232	JIP1	304	NSE	376	PTP gamma	448	STAT5b
17	ALOX5	89	COL6A1	161	GABRA5	233	Kallikrein 7	305	NT5E	377	PTP-MEG2	449	STAT6
18	alpha 2u-Globulin	90	COLEC10	162	GALNT2	234	KCNB2	306	NUAK1	378	PTPRM	450	STI1
19	ALPP	91	Complexin-1	163	gamma Catenin	235	KCNC1	307	Nucleostemin	379	PTPRU	451	STIM1
20	AMBP	92	Contactin-3	164	GATE-16	236	KIAA1967	308	NXPH3	380	PVRL1	452	STK3
21	AMH	93	COPZ1	165	GBL	237	Klotho beta	309	Oligodendrocyte Marker O1	381	QDPR	453	Substance P
22	Amphiphysin	94	CPEB3	166	GDF1	238	KMO	310	Oligodendrocyte Marker O4	382	Rab11A	454	SUMO3
23	AMPK beta 1	95	CPM	167	GDF7	239	KOR	311	Oncomodulin	383	Rab27a	455	SUSD2
24	ANG-2	96	CSNK1A	168	GDI1	240	KPNB1	312	OPRM1	384	RAB7A	456	Synaptotagmin-1
25	Angiogenin	97	CSNK1D	169	Gephyrin	241	Kynureninase	313	Osteopontin	385	RAC1	457	Syndecan-3
26	Annexin A11	98	CSNK1E	170	GLUD1	242	LAMC1	314	OV-6	386	RACK1	458	Syntaxin 1B
27	Annexin A2	99	CSNK1G	171	Glycine R	243	Laminin S	315	P20Sb3	387	Rad17	459	Syntaxin 7
28	ApoH	100	CSNK2B	172	GOLGB1	244	LC3B	316	p38 alpha	388	Raf-1	460	Syntaxin 8
29	ARC	101	CSRP1	173	GPLD1	245	LHX5	317	p70 S6 Kinase	389	Rap1A/B	461	Syntaxin BP1
30	ATF2	102	CXCR3	174	GPR64	246	LIPG	318	PABP	390	Rap2A/B	462	T Cell Receptor alpha Ch
31	ATF6	103	CXCR6	175	GPX2	247	Lipin 2	319	PAK4	391	RECQ4	463	TCEB2
32	ATG3	104	CYTL1	176	GPX4	248	LMAN1L	320	PAK6	392	REG4	464	TCP1 eta
33	ATM	105	DARC	177	GRB7	249	LMNA	321	Pannexin-1	393	Relaxin R1	465	Tenascin R
34	Axin-1	106	DARPP-32	178	GRP78	250	LOK	322	Park7	394	RELM gamma	466	TfR
35	B7-H4	107	DDC	179	GSK-3 alpha	251	LRPAP	323	PARL	395	RGM-B	467	TGN38
36	BAG4	108	DDT	180	H6PD	252	Lumican	324	Parvalbumin	396	RGM-C	468	TH
37	BAG6	109	DDX1	181	HABP2	253	Lysozyme	325	Paxillin	397	RHOG	469	Themis
38	BAMBI	110	DEFA6	182	HAO-1	254	LYVE1	326	PCBP2	398	RIBP	470	Thioredoxin-1
39	BarX1	111	DGK-gamma	183	HBB	255	MAD2L1	327	PCDH12	399	RIPK1	471	Thrombopoietin
40	BCHE	112	DGK-theta	184	HCLS1	256	MafB	328	PCK2	400	RKIP	472	TLR7
41	Beclin 1	113	DISC 1	185	HDAC2	257	MAP4K4	329	PCNA	401	RNASE4	473	TOP2B
42	beta-Actin	114	Dkk-1	186	HDAC4	258	Matrilin-4	330	PCSK9	402	RNF2	474	TOR
43	beta-I Tubulin	115	Dkk-2	187	HHEX	259	MBP	331	PDAP1	403	ROCK1	475	TRIM63
44	BMX	116	DOCK1	188	HIBADH	260	MCHR1	332	PDCD5	404	RPL10A	476	Troponin T
45	BNIP3L	117	DOT1L	189	HIF-2 alpha	261	M-CSF	333	PDCD6	405	RPL11	477	TRP14
46	BOK	118	DRAK2	190	Histamine H3 R	262	MDGA2	334	PDHX	406	RPL22	478	TRPV1
47	Brevican	119	Draxin	191	Histone H1.3	263	MDH1	335	PDK-1	407	RPLP2	479	TRXR1
48	CA14	120	DSC2	192	Histone H2AX	264	MDM2	336	PDX-1	408	RPS11	480	Trypsin 3
49	Cadherin-15	121	DYRK1A	193	HMGB1	265	MEK1	337	PDZK1	409	RPS19	481	Trypsin Pan
50	Cadherin-8	122	Dystroglycan	194	HMGN2	266	MEKK2	338	Perilipin-1	410	RPS25	482	TSC22
51	CALD1	123	EDN	195	HMOX1	267	MESDC2	339	PGAM2	411	RPS4X	483	TSH
52	Calretinin	124	EFEMP2	196	HN1	268	Metallothionein	340	PGK1	412	RPS6	484	TXNDC4
53	CaM Kinase II	125	EGLN1	197	hnRNP G	269	mGluR1	341	PGLS	413	RRAS2	485	UBASH3B
54	CaMKK alpha	126	EIF3D	198	hnRNP U	270	mGluR2/3	342	PGM1	414	RSK1	486	UBE2N
55	CapG	127	ELAVL1	199	HOMER1	271	mGluR5	343	PGRP-S	415	RSK2	487	UQCRB
56	CART	128	Endoglin	200	HP1BP3	272	MIB1	344	PIK3R1	416	RTN1-A	488	UROD
57	Cathepsin G	129	Endophilin A1	201	HPRT	273	MIOS	345	PIWIL2	417	RYK	489	VAP-B
58	Caveolin-1	130	Endorepellin	202	HS6ST3	274	MIP-1 beta	346	PKA RI beta	418	SC35	490	VE-Cadherin
59	CBP	131	ENSA	203	HSP90B1	275	MKP-3	347	PKC beta 1	419	SCGB3A1	491	Versican
60	CCBL1	132	EpCAM	204	HSPA2	276	MLK4	348	PKC gamma	420	SCGF	492	Vimentin
61	CCR2	133	EphA3	205	HSPB8	277	MN1	349	PKLR	421	SEC13	493	WNK1
62	CCR6	134	Ephrin-B3	206	IBP160	278	MPP5	350	PKN2	422	SECISBP2	494	WNK2
63	CCR8	135	ERBB4	207	ICAM-5	279	M-Ras	351	PLA2G2A	423	SEMA3F	495	WT1
64	CCR9	136	ERK1	208	IKK alpha	280	MSH6	352	PLC-beta 4	424	SENP8	496	WWOX
65	CD106	137	ERK2	209	IKK gamma	281	Musashi-1	353	PLC-gamma 1	425	Serpin A12	497	XPB
66	CD161	138	ERK4	210	IL-12 R beta 2	282	MyD88	354	Plexin A1	426	Serpin A3N	498	YAP1
67	CD164	139	FAIM3	211	IL-17F	283	MYHC	355	Plexin A2	427	Serpin A6	499	Yes
68	CD19	140	FANCD2	212	IL17RA	284	Myoglobin	356	Plexin A3	428	Serpin D1	500	ZBTB4
69	CD28	141	Fascin	213	IL-20RB	285	NAP1L1	357	Plexin B3	429	SerpinE2		
70	CD29	142	FASN	214	IL-21R	286	Nbs1	358	PLOD2	430	SerRS		
71	CD36	143	FBPase 1	215	IL-6R	287	NCAM2	359	PLTP	431	SGSH		
72	CD39L4	144	FCGR2B	216	IMPA1	288	NCOR1	360	PNPLA2	432	SHC1		

VII. Interpretation of Results:

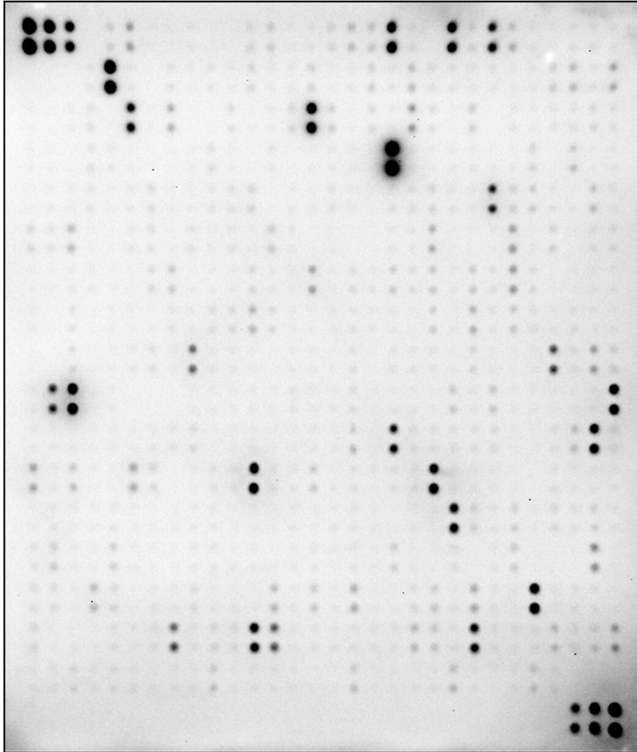
A. Explanation of Controls Spots

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.

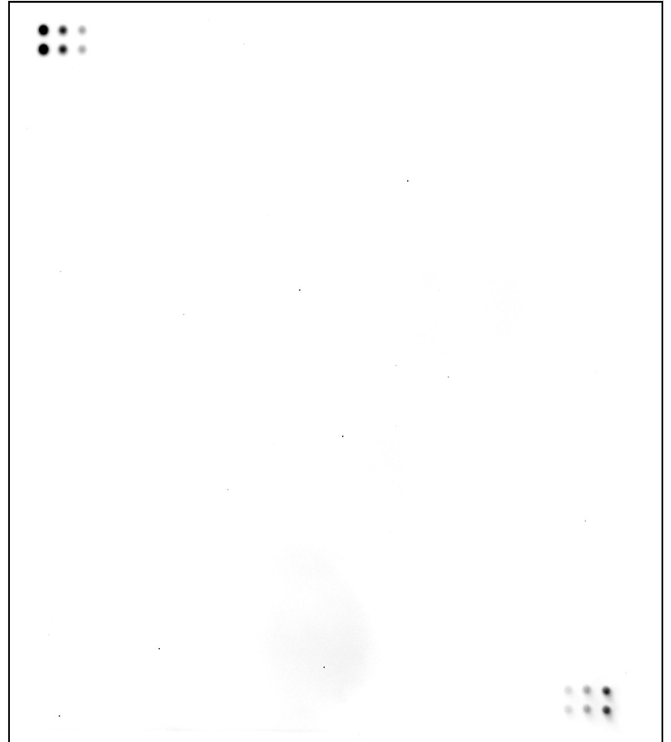
B. Typical Results

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

Rat 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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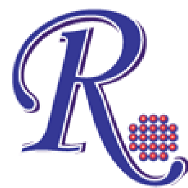

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