RayBio® Label-Based (L-Series) Rat L2 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-2-2 (2 Sample Kit)
AAR-BLM-2-4 (4 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

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Tel: +1-770-729-2992 or 1-888-494-8555 (Toll Free); Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

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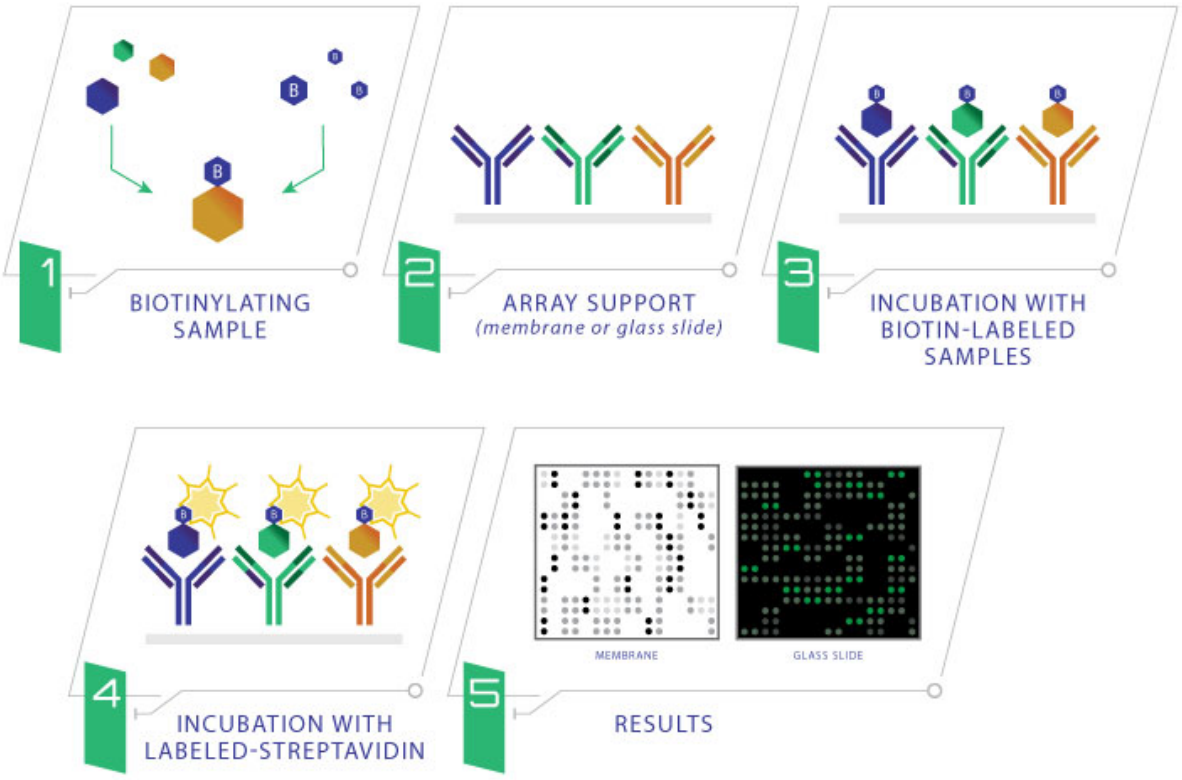
I. Introduction

Combining direct antigen-labeling technology with our vast library of array-validated antibodies, RayBiotech has created the largest commercially available antibody array to date. With the L-Series high density array platform, researchers can now detect thousands of proteins simultaneously, obtaining a broad, panoramic view of protein expression. Our newly expanded panel includes a wide variety of metabolic enzymes, structural proteins, epigenetic markers, neuroregulatory factors, in addition to our popular list of cytokines, growth factors, receptors, adipokines, proteases, and signaling proteins. Available on both glass slide and membrane formats, this array is ideally suited for biomarker discovery studies and exploratory screens.

The first step in using the RayBio® L-Series Antibody Array is to biotinylate the primary amine groups of the proteins in your sample (sera or plasma, cell culture supernatants, cell lysates or tissue lysates). The membrane arrays are then blocked, similar as a Western blot, and the biotin-labeled sample is added onto the membrane array which is pre-printed with capture antibodies and incubated to allow for interaction of target proteins. After incubation with HRP-Conjugated Streptavidin, the signals can be visualized by chemiluminescence.



II. Materials Provided

A. Storage Recommendations

Upon receipt, Box 1 should be stored at -20°C and Box 2 should be stored at 4°C. The kit must be used within 6 months from the date of shipment. After initial use, Blocking Buffer, Stop Solution, HRP-Conjugated Streptavidin, Detection Buffers C and D should be stored at 4°C to avoid repeated freeze-thaw cycles (may be stored for up to 3 months, Labeling Reagent, Item B should be fresh preparation before use). The Array Membrane should be kept at -20°C and avoid repeated freeze-thaw cycles (may be stored for up to 6 months).

Box 1 (store at -20°C):

ITEM	DESCRIPTION	2 MEMBRANE KIT	4 MEMBRANE KIT
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	1 vial (50 µl)
E	L-series Antibody Array Membranes	2 membranes	4 membranes
F	4X Blocking Buffer	1 bottle (30 ml)	1 bottle (30 ml)
I	500X HRP-Conjugated Streptavidin Concentrate	1 vial (100 µl)	1 vial (100 µl)
K	Detection Buffer C	1 bottle (10 ml)	2 bottles (10 ml)
L	Detection Buffer D	1 bottle (10 ml)	2 bottles (10 ml)
Other Kit Components: Plastic Sheets			

Box 2 (store at 4°C):

ITEM	DESCRIPTION	2 MEMBRANE KIT	4 MEMBRANE KIT
G	20X Wash Buffer 1 Concentrate	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer 2 Concentrate	1 bottle (30 ml)	1 bottle (30 ml)
	Labeling Buffer	1 bottle (30 ml)	2 bottles (30 ml/ea)
J-2	Spin Columns	4 columns	8 columns
N/A	Plastic Incubation Trays (w/lid)	2 trays	4 trays
N/A	2X Lysis Buffer	1 bottle (10 ml)	1 bottle (10 ml)

B. Additional Materials Required

- 2-5 ml tube, small plastic or glass containers
- 15 ml conical collection tubes
- Orbital shaker or oscillating rocker
- Kodak X-Omat™ AR film (REF 165 1454) and film processor or Chemiluminescence imaging system
- Pipettors, pipette tips and other common lab consumables
- Eppendorf tube

III. Overview and General Considerations

A. Preparation and Storage of Samples

1. Preparation of Cell Culture Supernatants
    1. Seed cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes.*
    2. Culture cells in complete culture medium for ~24-48 hours.**
    3. Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours.**,+
    4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as less than or equal 1 ml aliquots at -80°C until needed.
    5. If you want to use cell mass for inter-sample normalization, measure the total wet weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing densitometry signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can normalize between arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

        **The density of cells per dish used is dependent on the cell type. More or less cells may be required.*

        ***Optimal culture time may vary and will depend on the cell line, treatment conditions and other factors.*

+Bovine serum proteins produce detectable signals on the RayBio® L-Series Array in media containing serum concentrations as low as 0.2%. When testing serum-containing media, we strongly recommend testing an uncultured media blank for comparison with sample results.

2. Extracting Protein from Cells
    1. Centrifuging Cells
        a. Adherent Cells:
            i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.
            ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.
        b. Cells in Suspension: Pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 minutes.
    2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.
    3. Pipette up and down to resuspend cells and rock the lysates gently at 2-8°C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8°C.

        Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.
    4. Transfer lysates to a clean tube. Determining cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.
3. Extracting Protein from Crude Tissue
    1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O).
    2. Homogenize the tissue according to homogenizer manufacturer instructions.

3. Transfer extracts to microcentrifuge tubes and centrifuge for 20 minutes at 13,000 rpm (4°C).

   Note: If the supernatant appears to be cloudy, transfer the supernatants to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the supernatant is still not clear, store the lysate at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer supernatant to a clean tube and store at -80°C.

4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

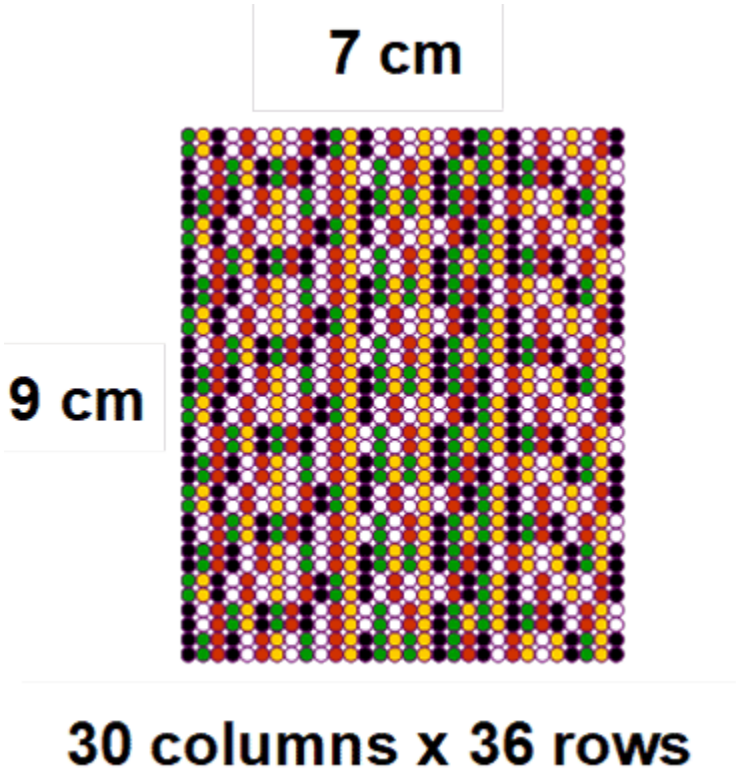
B. Handling the Array Membranes

- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

C. Incubations of Antibody Array

- Completely cover membranes with sample or buffer during incubation and cover the Plastic Incubation Tray with the lid to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Several incubation steps such as step 3 (sample incubation) or step 7 (HRP-Conjugated Streptavidin incubation) may be done at 4°C overnight.

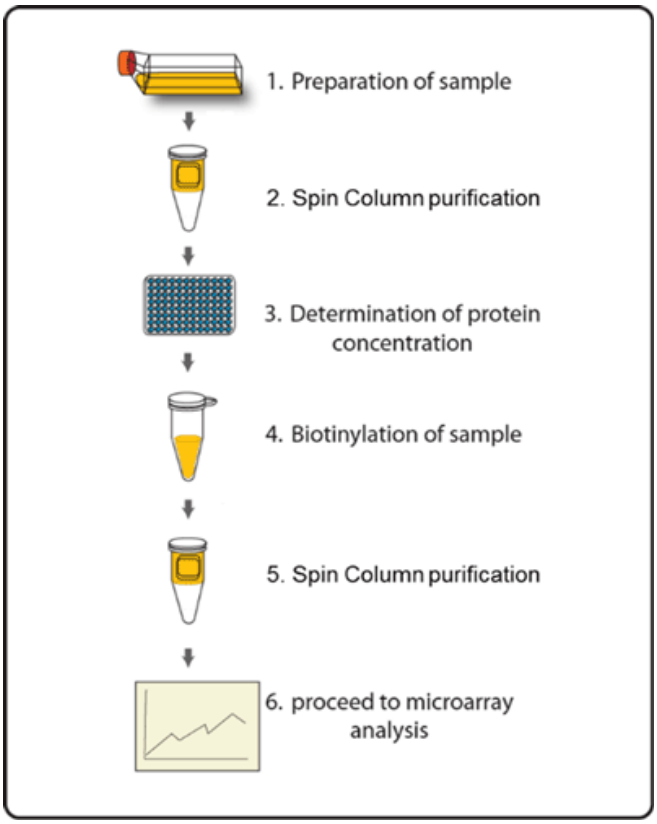
D. Layout of Array Membrane



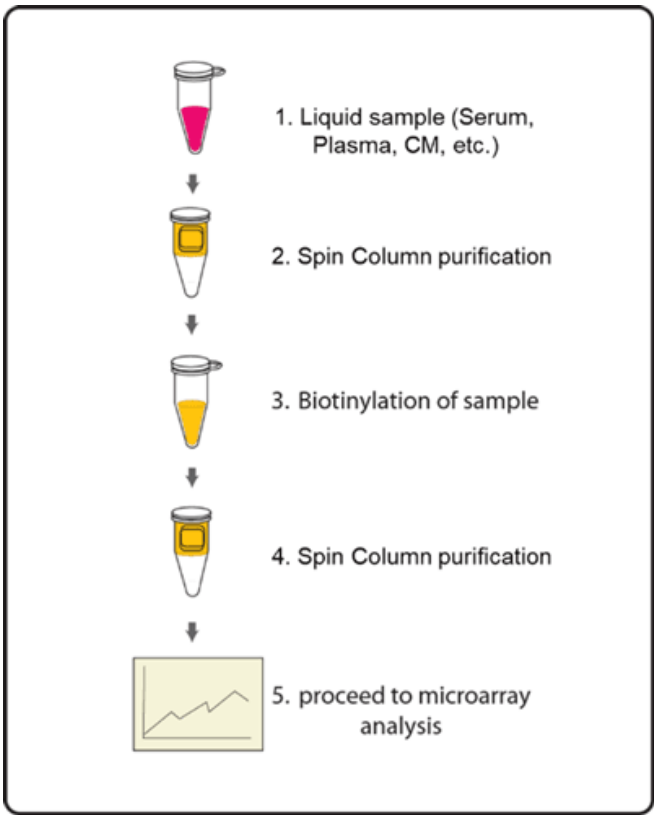
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



2. Serum, plasma, or Cell culture supernatants



A. Sample purification

Note: This step removes low molecular weight amine derivatives or unwanted buffer from samples to ensure quality biotinylation in Steps 5-7.

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).
2. Place the Spin column into a 15 ml conical collection tube, centrifuge at 1,000 x g for 3 minutes to remove the storage buffer. Discard the flow-through.
3. Wash the column three times with 1 ml labeling buffer each, centrifuge 1,000 x g for 3 minutes to remove the flow-through. Blot the bottom of the column to remove excess liquid, and transfer device to a new collection tube.
4. Apply sample on top of the resin within the next few minutes. Centrifuge at

1,000 x g for 3 minutes to collect the flow-through that contains sample. The recommended sample dilution as following:

- *Cell culture supernatant: 600 µl neat supernatant*
- *Serum/Plasma: 10 µl serum/plasma in 600 µl Labeling Buffer*
- *Cell/tissue lysate: 100 µg lysate in 500 µl Labeling Buffer*

Note: The maximal sample volume is 700 µl for each Spin Column. Do not load over 700 µl of sample into a Spin Column.

B. Biotin-Labeling the Sample

Note: Amines (e.g., Tris, glycine) and azides quench the biotinylation reaction. Avoid contaminating samples with these chemicals prior to biotinylation.

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.
6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.
    a. For labeling cell culture supernatants: Add 10 µl of Labeling Reagent into the sample tube (for 600 µl supernatant).
    b. For labeling serum or plasma: Add 10 µl of Labeling Reagent into the sample tube (for 10 µl serum/plasma *in 600 µl labeling buffer*).
    c. For labeling cell or tissue lysates: Add 5 µl of 1X Labeling Reagent into the sample tube (for 100 µg lysate *in 500 µl labeling buffer*).
    d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

    Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If more or less amount sample is labelled, adjust this volume proportionally.
7. Add 5 µl Stop Solution (Item D) to each sample tube. Using a new spin column, repeat Steps 1-4 of section A. Sample Purification to remove the

excess non-reacted biotin reagent from each sample.

Note: Biotinylated samples can be stored at -20°C or -80°C until you are ready to proceed with the assay.

C. Blocking and Incubations

8. Place each membrane printed side up into a Plastic Incubation Tray (provided). 1 membrane per tray.

   Note: The printed membrane will have a "-" mark in the upper left corner of the membrane.

   Note: Up to 4 membranes can be incubated together within one tray with proportional amount of reaction buffer. Rotate the membrane sequence at least once during sample incubation if more than one membrane is incubated in one tray.

9. Dilute 4X Blocking Buffer (Item F) with deionized or distilled water to prepare the 1X Blocking Buffer. Add 6 ml of 1X Blocking Buffer to each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour.

10. Aspirate the Blocking Buffer from each tray. Add 6 ml of diluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

    Note: It is recommended to use 10-20 folds diluted biotin-labeled culture supernatant, 10-20 folds diluted biotin-labeled serum/plasma, 100 folds diluted biotin-labeled cell/tissue lysate, or 10-20 folds for other body fluid. Dilute sample using 1X Blocking Buffer. The optimal concentration of sample used will depend on the abundance of target proteins. The samples can be concentrated if the overall signals are too weak. If the overall signals are too strong, the sample can be diluted further.

    Note: Incubation may be done at room temperature with gentle shaking for 2 hours or overnight at 4°C.

11. Dilute 20X Wash Buffer 1 (Item G) with deionized or distilled water to prepare the 1X Wash Buffer 1. Aspirate the samples from each tray and then wash by adding 20 ml of 1X Wash Buffer I at room temperature with gentle shaking (5 min per wash). Repeat the wash 2 more times for a total of 3 washes.

12. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 (Item H) with deionized or distilled water to prepare the 1X Wash Buffer 2. Wash 3 times with 20 ml of 1X Wash Buffer 2 at room temperature with gentle shaking.
13. Aspirate the 1X Wash Buffer 2 from each tray.
14. Prepare the HRP-Conjugated Streptavidin. Briefly spin down the tube containing the 500X HRP-Conjugated Streptavidin (Item I) immediately before use. Dilute the 500X HRP-Conjugated Streptavidin with 1X Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. Pipette up and down to mix gently. Add 6 ml of 1X HRP-Conjugated Streptavidin to each membrane.

    Note: Ensure that the vial containing the 500X HRP-Conjugated Streptavidin is mixed well before use, as precipitation can form during storage.
15. Incubate at room temperature with gentle shaking for 2 hours.

    Note: incubation may be done overnight at 4°C.
16. Wash as directed in steps 11 through 13.

D. Detection

Note: Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.

17. For detection of 2 membranes, add 4.2 ml of Detection Buffer C and 4.2 ml of Detection buffer D into a tube and mix both solutions. Drain off excess wash buffer. Place membrane antibody side up (There is a "-" symbol on the top left corner of each membrane) on a clean plastic plate or its cover (provided in the kit). Pipette 4 ml of the mixed Detection Buffers onto each membrane and incubate at room temperature for 2 minutes with gentle shaking. Ensure that the detection mixture is evenly covering the membrane without any air bubbles.
18. Gently place the membrane with forceps (antibody side up) on a plastic sheet (provided) and cover the membrane with another plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane. Work as quickly as possible.
19. The signal can be detected directly from the membrane using a chemiluminescence imaging system or by exposing the array to x-ray film (we recommend using Kodak X-Omat™ AR film) with subsequent development.

Expose the membranes for 40 seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce the exposure time (e.g., 5-30 seconds). If the signals are too weak, increase the exposure time (e.g., 5-20 min or overnight) or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

20. Save membranes at -20°C to -80°C for future reference.

V. Antibody Array Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
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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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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	11b-HSD1	73	CD1d1	145	DYRK2	217	GLRX3	289	IPP2	361	Notch-1	433	SEMA4D
2	14-3-3 epsilon	74	CD200	146	DYRK3	218	Glyoxalase 1	290	Islet-1	362	Notch-2	434	SEMA5A
3	14-3-3 eta	75	CD22	147	ECM1	219	Glyoxalase 2	291	Jagged 1	363	Notch-3	435	SEMA7A
4	14-3-3 sigma	76	CD226	148	EEA1	220	Glypican 3	292	JAM-A	364	NPC2	436	Serpin F2
5	14-3-3 theta	77	CD27	149	EFNA1	221	gp130	293	JAM-C	365	NRAGE	437	SerpinA1
6	4-1BB	78	CD276	150	EFNB1	222	GPT	294	JNK1	366	Nrf2	438	SerpinE1
7	A2B5	79	CD300f	151	EGF	223	Gpt2	295	JNK2	367	NRXN1 beta	439	SerpinF1
8	ACACA	80	CD300LG	152	EGFR	224	GPX1	296	KDR	368	Olfactomedin-1	440	SH2B1
9	ACTC1	81	CD31	153	eIF5A	225	GPX3	297	Keap1	369	OLR1	441	SHIP2
10	Actin	82	CD34	154	EMP	226	Granzyme B	298	Kirrel3	370	Osteocalcin	442	SHP-1
11	Activin R2A	83	CD38	155	ENO1	227	GRB2	299	KLKB1	371	OX40	443	SIGNR1
12	ADAM10	84	CD39L1	156	Eotaxin	228	GRIN2A	300	KNG1	372	p27	444	SIRP alpha
13	ADAMTS1	85	CD4	157	EphA5	229	GRK1	301	LAIR1	373	p38 gamma	445	SLAMF1
14	Adiponectin	86	CD47	158	EphB1	230	GRK2	302	LAR	374	p53	446	SLC4A1
15	aFGF	87	CD48	159	EphB6	231	GRK5	303	LAYN	375	p55PIK	447	Slit3
16	Agrin	88	CD5L	160	Ephrin-A2	232	GRO alpha	304	LDHA	376	PAK1	448	Smad 3
17	AIF	89	CD6	161	Ephrin-B2	233	GRP75	305	Legumain	377	PAK7	449	Smad 7
18	AK1	90	CD63	162	ER alpha	234	HAAO	306	Leptin	378	Pax7	450	SMC1
19	ALCAM	91	CD68	163	ERBB2	235	HABP1	307	Leptin Receptor	379	P-Cadherin	451	Sortilin
20	ALK-7	92	CD79B	164	ERBB3	236	HGF	308	LIF	380	PCDH-17	452	SOST
21	Alpha-Actinin 1	93	CD8 alpha	165	Erythropoietin	237	HIF-1 alpha	309	LIFR	381	PCK1	453	SOX1
22	Alpha-Synuclein	94	CD83	166	Ets-1	238	HO-2	310	LILRA5	382	PDGF-BB	454	SOX10
23	Ameloblastin	95	CD86	167	Ezrin	239	HPRG	311	LILRC2	383	PDGFRA	455	SOX2
24	AMPK alpha 2	96	CD93	168	F2	240	HPX	312	Lipocalin-2	384	Pentraxin 2	456	SP-D
25	Androgen R	97	CDC25B	169	F3	241	HSP20	313	LMW-PTP	385	Peroxiredoxin 6	457	Src
26	ANGPT1	98	CDC37	170	FABP1	242	HSP27	314	LPHN3	386	PFKM	458	STAT3
27	Annexin A1	99	CDH1	171	FABP2	243	HSP40	315	LRP-4	387	PGC	459	Syndecan-2
28	Annexin A4	100	CDH2	172	FABP3	244	HSP60	316	LTBR	388	pIgR	460	Syntaxin 1A
29	Annexin A7	101	CDNF	173	FABP4	245	HSP70	317	LTF	389	PIM2	461	TAFA5
30	Annexin V	102	CES1	174	FABP5	246	HSP90	318	Lyn	390	PKA C a/b	462	Talin1
31	APE	103	CF XIV	175	FAK	247	HSPA8	319	MAG	391	PKC	463	TCK-1
32	APLP-1	104	CHMP2B	176	FCAR	248	HSPH1	320	Matrilin-3	392	PKC a	464	TC-PTP
33	APRIL	105	Chordin	177	FCGR1	249	HtrA2	321	MBL-2	393	PKC i/l/z	465	TDP-43
34	Arginase 1	106	CIB1	178	FETUB	250	IDS	322	MCAM	394	PKM2	466	TF
35	ART4	107	CLEC4A2	179	FGF-12	251	IFNA5	323	MCP-3	395	PLAUR	467	TGF-beta RIII
36	ASAH2	108	CLEC4B2	180	FGF-21	252	IFN-alpha	324	MEK2	396	Plexin A4	468	TGM2
37	B3GNT2	109	Clusterin	181	FGFR4	253	IFN-gamma	325	MIF	397	PON3	469	THBD
38	BAFF	110	CNTF	182	Fgr	254	IFN-gamma R2	326	MIG	398	POR	470	Thioredoxin-2
39	BAK	111	CO5	183	Fibromodulin	255	IGF-1	327	MIP-1 alpha	399	PP2A CS	471	TIE-2
40	BCAM	112	COLEC12	184	FKBP12	256	IGFBP-5	328	MIP-3 beta	400	PP2C alpha	472	TIM-1
41	Bcl-10	113	Complexin-2	185	FKBP12.6	257	IGSF8	329	MKK6	401	PPA1	473	TNF alpha
42	Bcl-2	114	Contactin-1	186	FKBP13	258	IkB-beta	330	MMP-2	402	PPP2R4	474	TNF-R1
43	BCL-W	115	Contactin-2	187	FKBP25	259	IKK	331	MMP-8	403	PRDX 2	475	TNFRSF11A
44	Bcl-xL	116	Contactin-4	188	FKBP51	260	IL-1 beta	332	MMP-9	404	PRDX1	476	TNFSF9
45	beta 2-M	117	Cortactin	189	FKBP52	261	IL-1 RA	333	MOG	405	PRDX4	477	Tollip
46	beta IG-H3	118	CPA1	190	FLIP	262	IL-10	334	MP1	406	Pref-1	478	TPT1
47	bFGF	119	CPA2	191	FLT1	263	IL-11 R alpha	335	MPO	407	PRL-3	479	TRAF-2
48	BID	120	CPB1	192	Flt-3 Ligand	264	IL-12 p70	336	MST1	408	PRL8A4	480	TRAF-3
49	BIK	121	CRELD1	193	Follistatin	265	IL-13 Ra2	337	NCAM-1	409	PROCR	481	Transgelin
50	BLVRB	122	CRELD2	194	FOLR1	266	IL-15 Ra	338	NCR3	410	Prolactin	482	TREM-1
51	BMP-2	123	CrkL	195	FRK	267	IL-17 RC	339	NEDD4	411	Properdin	483	TRHDE
52	BMP-7	124	CRP	196	FRS2	268	IL-18	340	NEDD8	412	PSAP	484	TrkA
53	B-raf	125	CRYAB	197	GABRA4	269	IL-18 BPc	341	Nephrin	413	PSMA1	485	TrkB
54	BST1	126	CSF1R	198	GAD1	270	IL1R1	342	Nestin	414	PSMA2	486	TrkC
55	BTLA	127	CTACK	199	Galectin-1	271	IL1R2	343	Netrin-1	415	PTK7	487	TWEAK R
56	C4.4A	128	CTGF	200	Galectin-3	272	IL-2	344	Neurexophilin-1	416	PTP1B	488	UCH-L1
57	Cadherin-4	129	CTHRC1	201	Galectin-4	273	IL-2 Ra	345	Neuritin	417	PVR	489	UCH-L3
58	CADM3	130	CTLA4	202	GAPDH	274	IL-2 RG	346	Neurocan	418	PVRL2	490	UNC5H1
59	Calcineurin A	131	Cubilin	203	Gas 1	275	IL-21	347	Neurofascin	419	RalA	491	UNC5H2
60	Calcineurin B	132	CXCL10	204	GDF-3	276	IL-22	348	Neurogranin	420	RALT	492	VAMP-2
61	Caspr 2	133	CXCL16	205	GDF-8	277	IL-23 p19	349	Neuroligin-1	421	RANTES	493	VHR
62	Catalase	134	Cyclophilin A	206	GDNF	278	IL-31	350	Neuroligin-2	422	RBBP4	494	Vinculin
63	Cathepsin B	135	Cyclophilin B	207	GFAP	279	IL-4	351	Neuropilin-1	423	RBP4	495	VSIG1
64	Cathepsin C	136	Cystatin C	208	GFRA1	280	IL-4 R	352	Neuroplastin 65	424	Reg III	496	WFDC2
65	Cathepsin E	137	Cytochrome-C	209	GFRA2	281	IL-6	353	NFATC3	425	Reg3B	497	Wnt5a
66	Cathepsin L	138	Decorin	210	GFRA3	282	IL-7	354	NF-L	426	Renin 1	498	XIAP
67	Cathepsin X	139	DEP-1	211	GGT1	283	IL-7 Ra	355	NM23-H1/H2	427	RHD	499	XPNPEP2
68	Caveolin-2	140	DGK-epsilon	212	GH	284	IL-9	356	nNOS	428	ROBO1	500	Zyxin
69	CCK-A R	141	DHFR	213	GIT1	285	IL-9 R	357	NNUP85	429	ROCK2		
70	CCL26-Like	142	Dkk-3	214	GITR	286	ILK	358	Noggin	430	SDC1		
71	CD13	143	DLL1	215	GLA	287	ILKAP	359	Nogo-A	431	Secretagogin		
72	CD14	144	DOK7	216	GLG1	288	IMPDH1	360	Nope	432	SEMA4C		

VII. Interpretation of Results:

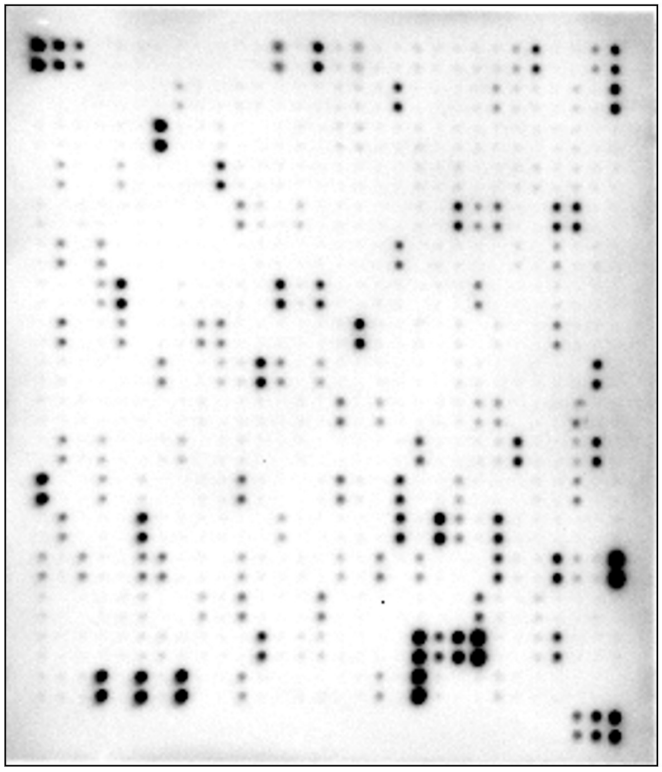
A. Explanation of Controls Spots

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.

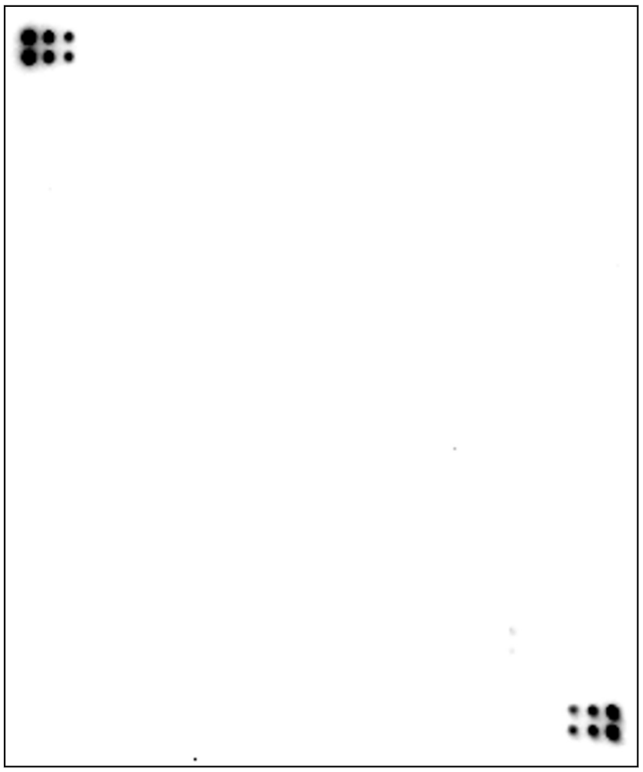
B. Typical Results

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

Rat Plasma



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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