RayBio® Label-based (L-Series) Human Antibody Array L-3 Membrane Kit

User Manual (Revised August 31, 2018)

For the simultaneous detection of the relative expression of 500 human proteins in cell/tissue lysates and serum/plasma.

L-Series Human Antibody Array L-3:
For Cell/Tissue and Serum/Plasma
Cat# AAH-BLM-3B-2 (2 Sample Kit)
Cat# AAH-BLM-3B-4 (4 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech, Inc.
the protein array pioneer company

Your Provider for Excellent Protein Array Systems and Services

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RayBiotech, Inc

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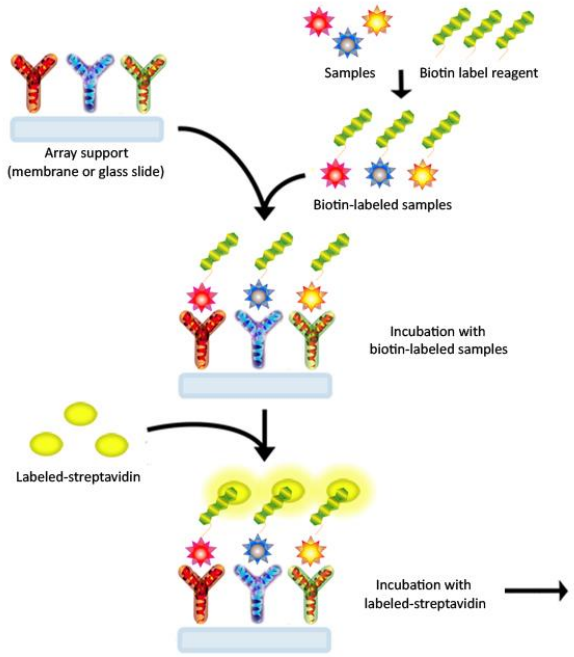
I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the L-Series Antibody Array L-3, researchers can now obtain a broad, panoramic view of protein expression. The expression levels of 500 human target proteins can be simultaneously detected, including extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins in cell culture supernatants, cell lysate, tissue lysate, serum and plasma.

The first step in using the Human L-3 array is to biotinylate the primary amines of the proteins in the sample. The membrane arrays are then blocked, similar to a Western blot, and the biotin-labeled sample is added onto the membrane array, which is pre-printed with capture antibodies. The membrane is then 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, the Blocking Buffer, Stop Solution, HRP-Conjugated Streptavidin and 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 prepared fresh each time before use. The Array Membrane should be kept at -20 °C and repeated freeze-thaw cycles should be avoided (may be stored for up to 6 months).

RayBio® L-Series Human Antibody Array L-3

Box 1 (store at -20 °C):

ITEM	DESCRIPTION	AAH-BLM-3B-2	AAH-BLM-3B-4
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio L-Series Human L-3 Antibody array membrane	2 membranes L-3	4 membranes L-3
F	Blocking Buffer	2 vials (30 ml/ea)	4 vials (30 ml/ea)
I	500X HRP-Conjugated Streptavidin Concentrate	1 vial (100 µl)	2 vials (100 µl/ea)
K	Detection Buffer C	1 vial (10 ml)	2 vials (10 ml/ea)
L	Detection Buffer D	1 vial (10 ml)	2 vials (10 ml/ea)
Other Kit Components: Plastic Sheets			

Box 2 (store at 4 °C):

ITEM	DESCRIPTION	AAH-BLM-3B-2	AAH-BLM-3B-4
A-2	Dialysis Vials	2 vials	4 vials

G	20X Wash Buffer 1 Concentrate	1 vial (30 ml)	1 vial (30 ml)
H	20X Wash Buffer 2 Concentrate	1 vial (30 ml)	1 vial (30 ml)
J-2	Spin Columns	2 columns	4 columns
N/A	Plastic Incubation Trays (w/lid)	2 trays	4 trays
M	Floating Dialysis Rack	1 rack	
N/A	2X Lysis Buffer*	1 bottle (10 ml)	

* Only need if running cell or tissue lysates

B. Additional Materials Required

- 1X PBS, pH=8.0
- Shaker
- 2 - 5 ml tube
- 50 ml conical collection tubes
- Distilled water
- Kodak X-Omat™ AR film (REF 165 1454) and film processor or Chemiluminescence imaging system
- Large beaker
- Stir plate
- Eppendorf tubes

III. Overview and General Considerations

A. Preparation and Storage of Samples

1. Extracting Protein from Cells

1) Centrifuge cells.
    a. Adherent cells:

i. Remove supernatant from the cell culture and wash cells gently two times with cold 1X PBS taking care not to disturb the cell layer.
ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells. Proceed to b. Cells in Suspension.

b. Cells in Suspension:
i. Pellet the cells by centrifuging using a microcentrifuge tube 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 deionized or distilled water). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.

3) Pipette up and down to resuspend the cells, and rock the lysates gently at 2-8 °C for 30 minutes. Transfer the lysates to microfuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8 °C.

Note: If the supernatant appears to be cloudy, transfer the supernatant 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 -80°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. Determine the cell lysate concentration using a total protein assay (BCA Protein Assay

Kit, Pierce, Prod# 23227). Aliquot the lysates and store at – 80°C.

2. Extracting Protein from Crude Tissue

1) Transfer approximately 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water).

2) Homogenize the tissue according to the homogenizer manufacturer's instructions.

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

   Note: If the supernatant appears to be cloudy, transfer the supernatant 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 -80°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4) Transfer the supernatant to a clean tube and store at – 80°C.

B. Handling Array Membranes

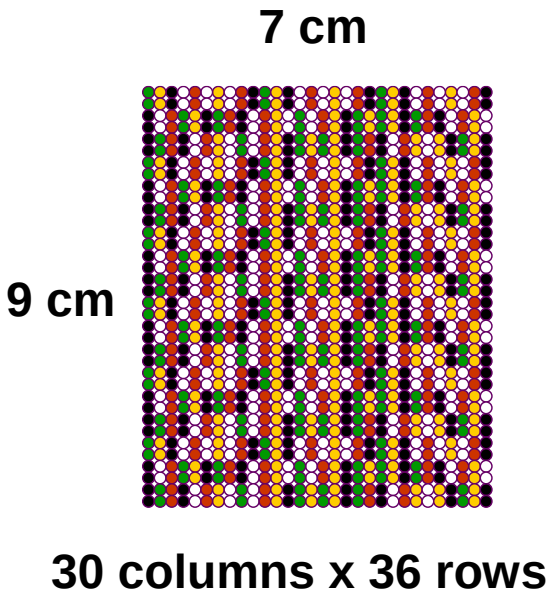
1) Always use forceps to handle membranes and grip the membranes by the edges only.
2) Never allow membranes to dry during the experiment.
3) Avoid touching membranes with hands or any sharp tools.

C. Incubations and Washes

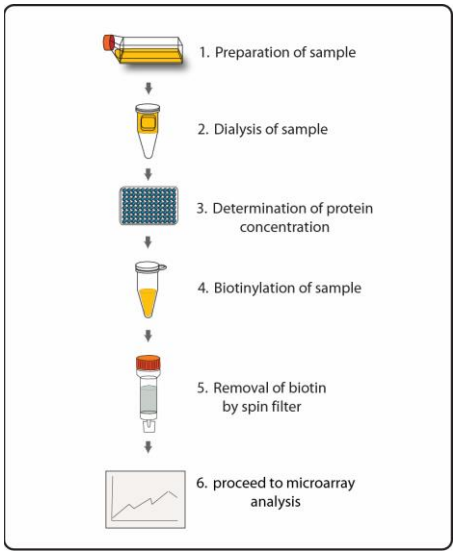
1) Completely cover membranes with sample or buffer during incubation and cover the Plastic Incubation Tray with the lid to avoid drying.
2) Avoid foaming during incubation steps.
3) Perform all incubation and wash steps under gentle rotation.
4) Several incubation steps such as step 10 on page 12 (sample incubation) or step 14 on page 13 (HRP-Conjugated Streptavidin incubation) may be done at 4 °C overnight.

IV. Protocol

Layout of L-3 Array Membrane



Assay Diagram*



* For serum or plasma, refer to Steps 2, 4, 5 and 6 only.

A. Dialysis of Sample

Note: Samples must be dialyzed prior to biotin-labeling (Steps 5–7).

1) Prepare enough dialysis buffer (1X PBS, pH=8.0) for all dialysis steps herein and after. To prepare 1 L dialysis buffer, dissolve 0.2 g KCl, 8 g NaCl, 0.2 g KH_2PO_4 and 1.15 g Na_2HPO_4 in 800 ml ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 ml with ddH_2O.

2) Load each sample into a separate Dialysis Vial (Item A-2). Loading volumes are as follows: 0.1-0.2 ml cell or tissue lysate (~ 1-2 mg/ml total protein) per vial for dialyzing; 30 µl serum or plasma + 120 µl dialysis buffer (5-fold dilution) per vial for dialyzing. Carefully place all Dialysis Vials into the Floating Rack.

Note: If the sample appears to be cloudy, transfer the supernatant to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C.

3) Place the Floating Rack into ≥500 ml dialysis buffer in a large beaker. For more than 2 samples, make certain to use at least 300 ml dialysis buffer for each sample (more buffer will improve the efficiency of dialysis). Place beaker on a stir plate and dialyze for at least 3 hours at 4˚C, occasionally stirring the dialysis buffer gently. Then exchange the dialysis buffer with fresh buffer and repeat dialysis for at least 3 hours at 4˚C. Transfer the dialyzed samples into clean eppendorf tubes. Centrifuge dialyzed samples for 5 minutes at 10,000 rpm to remove any particulates or precipitates, and then transfer and combine each sample into one clean eppendorf tube. Mix well by gently pipetting.

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

Note: Determine the total protein concentration for cell or tissue lysates after the dialysis procedure (Step 3). RayBiotech recommends a BCA total protein assay (e.g. Pierce BCA Protein Assay Kit, cat# 23227).

B. Biotin-Labeling of Sample

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

4) Immediately before use, prepare 1X Labeling Reagent by briefly centrifuging the Labeling Reagent vial (Item B) and adding 500 µl 1X PBS (pH 8.0) into the vial. Pipette up and down or vortex briefly to dissolve the lyophilized reagent.

5) Add 1X Labeling Reagent to dialyzed samples.

   a. For labeling cell or tissue lysates: transfer 90 µg total protein (e.g., 45 µl of 2 mg/ml) cell or tissue lysates into a tube and add enough 1X PBS for a total volume of 260 µl. Then add 10 µl 1X Labeling Reagent.
   b. For labeling serum or plasma: Add 65 µl of 1X Labeling Reagent Solution into a new tube containing 105 µl dialyzed serum or plasma sample and 80 µl 1X PBS.

Note: To normalize serum/plasma concentrations during biotinylation, measure the sample volume before and after dialysis. Then adjust the volumes of the dialyzed serum/plasma and 1X PBS Buffer to compensate. For example, if the sample volume increases 1.4 fold (from 150 µl to *210 µl, see "Dialysis of Sample" on page 11) after dialysis, then use 1.4 fold more*

*serum/plasma (1.4*105 µl = 147 µl) and reduce the added 1X PBS to 38 µl (80 µl - 42 µl = 38 µl).*

6) Incubate the reaction solution at room temperature for 30 minutes with gentle rocking or shaking. Mix the reaction solution by gently tapping the tube every 5 minutes.

7) Add 5 µl Stop Solution (Item D) into each reaction tube and then use the Spin Column (Item J-2) as follows to remove any unbound biotin.

a) Twist off the bottom closure of the Spin Column and loosen the cap (but keep the cap on). Place the Spin Column into a 16 ml conical collection tube for J-2.

b) Centrifuge the Spin Column at 1,000 x g for 3 minutes to remove the storage solution.

Note: The resin should appear compacted after centrifugation.

c) Add 1 ml 1X PBS (pH=8.0) into the Item J-2, Spin Column and centrifuge at 1,000 x g for 3 minutes to remove the 1X PBS. Repeat an additional 2 times to wash the Spin Column.

d) Place the Spin Column in a new 15 ml conical collection tube and slowly load biotinylated cell/tissue or serum/plasma to the center of the compact resin bed.

Note: The maximal sample volume is 700 ul for J-2 each Spin Column. Do not load over maximal sample volume sample into a Spin Column.

e) Centrifuge the Spin Column at 1,000 x g for 3 minutes. The sample should filter through the resin and deposit into the 15 ml conical collection tube. Store the sample at -80 ^{0}C until you are ready to proceed with the assay. Discard the Spin Column after use.

C. Blocking and Incubation

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

Note: The printed membrane will have a "3" mark for all L-3 membranes in the upper left corner of the membrane.

9) Add 4 ml of Blocking Buffer (Item F) 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 4 ml of diluted* or undiluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

* It is recommended to use 4 ml of 80-fold diluted biotin-labeled cell/tissue lysate or 4 ml 70 fold diluted biotin-labeled serum/plasma. Dilute sample using Blocking Buffer.

Note: 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 Blocking Buffer to prepare the

1X HRP-Conjugated Streptavidin. Pipette up and down to mix gently. Add 8 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 ^{o}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 ("1" or "2" symbol is marked 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

RayBio® L-Series Human Antibody Array L-3 Map – if needed, larger versions of these maps can be obtained by contacting technical support at 770-729-2992 or by emailing techsupport@raybiotech.com.

RayBio® L-Series Human Antibody Array L-3 Map

P-1a	P-2a	P-3a	N	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
P-1a	P-2a	P-3a	N	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
N	N	N	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
N	N	N	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
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B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

RayBio® L-Series Human Antibody Array L-3 List

number	name	number	name	number	name	number	name	number	name
1	Pos 1a	61	Alpha Fodrin	121	BCHE	181	Cathepsin A	241	Col6A2
2	Pos 2a	62	alpha Glucosidase II	122	Bcl-w	182	Cathepsin G	242	COL9A3
3	Pos 3a	63	alpha -Synuclein	123	BCOR	183	Cathepsin H	243	COLEC10
4	Neg	64	alpha Tubulin	124	beta 1 Spectrin	184	Cathepsin X/Z/P	244	Collagen I a1
5	14-3-3 beta	65	AlphaA Crystallin/CRYAA	125	beta B1 Crystallin/CRYBB1	185	CBS	245	Collagen III
6	14-3-3 epsilon	66	ALS	126	beta -I Tubulin	186	CCDC126	246	Collagen IVa6
7	14-3-3 eta	67	Als2	127	beta III Tubulin/CUBB3	187	CCDC25	247	Collagen IX
8	14-3-3 gamma	68	ALS+B62:B1212CR1	128	BID	188	CCT3	248	Collagen V
9	14-3-3 sigma	69	Aminoacylase	129	BIN2	189	CD109	249	Collagen VI
10	14-3-3 theta	70	Androgen Receptor	130	BIRC6	190	CD133	250	Collagen X
11	14-3-3 zeta	71	ANGPTL6	131	BLMH	191	CD155	251	Collagen XV alpha 1
12	53BP1	72	ANGPTL8	132	BLVRB	192	CD157	252	COMP
13	67LR	73	ANK	133	BMP-1	193	CD16	253	Complement Factor B
14	ABAT	74	Ankrd26	134	BPGM	194	CD21	254	Contactin-3
15	ABCF1	75	Annexin A1	135	BPIFB1	195	CD32	255	COPS8
16	ABI3BP	76	Annexin A2	136	BPIL1	196	CD35	256	Corneodesmosin
17	ACAA1	77	Annexin A6	137	BRCA 2	197	CD39L4	257	Coronin 3
18	ACAA2	78	Annexin V	138	BRD2	198	CD41	258	Cortactin
19	ACACA	79	ANP	139	Brevican	199	CD42b	259	COTL1
20	Acetyl-CoA acetyltransferase/ACAA	80	Antithrombin III	140	Brg1	200	CD48	260	CPE
21	ACLP	81	APA	141	BRSK1	201	CD5L	261	CPEB3
22	ACLY	82	APLP-1	142	BTD	202	CD9	262	CPM
23	Aconitase 1	83	APM2	143	BTF3	203	CD98	263	CPN1
24	ACTBL2	84	Apo (a)	144	C 1q	204	CDA	264	CPNE3
25	ACTC1	85	APOA1BP	145	C 1q S	205	CDC5L	265	CPS1
26	Actinin alpha 1	86	Apolipoprotein F	146	C1QB	206	CDK2	266	Creatine Kinase MM/CKMM
27	ADAMDEC1	87	Apolipoprotein L 1	147	C1qR1	207	CEACAM-8/CD66b	267	CRF21
28	ADAS	88	Apolipoprotein L 2	148	C1RL	208	CECR1	268	CRHBP
29	ADH1B	89	ARFBP1	149	C1s	209	CENPF	269	CrkL
30	ADH1C	90	ARFGEF3	150	C3orf75	210	CEP57	270	CRMP2
31	Neg	91	Argininosuccinate Lyase/ASL	151	C4.4A	211	CES1	271	CRTAC1
32	Neg	92	ArgRS	152	C4BPA	212	CETP	272	CS
33	Neg	93	ARP19	153	C5b-9	213	Cezanne	273	Ctip2
34	ADH4	94	Arp2	154	C6 -N-t	214	CFHR 1	274	Cux2
35	ADH5	95	ARP2/3	155	C8G	215	CFHR4	275	Cyclophilin A
36	ADM	96	Arp3	156	C9orf40	216	CFHR5	276	Cyclophilin B
37	Advillin-N-t	97	ARPC2	157	CA1	217	CFI	277	Cystatin D
38	AFG3L2	98	ARPC3	158	CA150	218	CFL1	278	Cystatin E/M
39	AGA	99	ART3	159	CA2	219	CFVII	279	Cystatin S
40	Aggrecan	100	ARTS1	160	CA3	220	CHC17	280	Cystatin SN
41	AGXT	101	ARX	161	CACNB4	221	Chitobiase	281	Cysteine-rich Protein 1
42	AHNAK	102	ASH2L	162	CAD	222	Chitotriosidase	282	CYTL1
43	Ahsp	103	ASGR2	163	Cadherin 22	223	CHORDC1	283	Cytochrome b5
44	AIF	104	ASK1	164	Cadherin-6	224	CHREBP	284	Cytochrome c
45	AK2	105	Aspartate Aminotransferase /AST	165	Caldesmon/CALD1	225	Chromogranin B	285	Cytokeratin 1
46	AKAP9	106	Aspartyl Aminopeptidase/DNPEP	166	CALML5	226	Chromogranin C	286	Cytokeratin 10
47	AKR1B1	107	ASXL1	167	Calmodulin	227	CIP29	287	Cytokeratin 13
48	AKR1C3	108	ATBF1/ZFHX3	168	Calpain 1	228	CKB	288	Cytokeratin 14
49	AKR7A2	109	ATP5A	169	Calpain S1	229	CLIC1	289	Cytokeratin 15
50	ALAD	110	ATP5O	170	Calpastatin	230	CLIC4	290	Cytokeratin 16
51	Alanine Transaminase/ALT	111	ATPB	171	Calretinin	231	CLIP170-N-t	291	Cytokeratin 17
52	Alcohol Dehydrogenase/ADH	112	B3GNT2	172	Calumenin	232	CL-P1	292	Cytokeratin 20
53	Aldehyde Oxidase 1/AOX1	113	B4GalT1	173	CAP1	233	CLPS	293	Cytokeratin 3
54	ALDH16A1	114	B7-H2	174	CapG	234	CLTA	294	Cytokeratin 4
55	ALDH1A1	115	B7-H3	175	CAPZA1	235	CNN2	295	Cytokeratin 5
56	ALDH9A1	116	BAD	176	Carboxypeptidase B2/CPB2	236	CNOT1	296	Cytokeratin 9
57	ALKP	117	Band 3	177	CARHSP1	237	CO4A2	297	D4 GDI
58	ALP	118	BASP1	178	Caspase-14	238	COG4	298	DAK
59	alpha 1,2 Mannosidase IA	119	Bassoon	179	Catalase	239	COL19A1	299	DAN
60	alpha Actinin 4	120	BAZ2B	180	Cathelicidin	240	COL4A3	300	DARS2

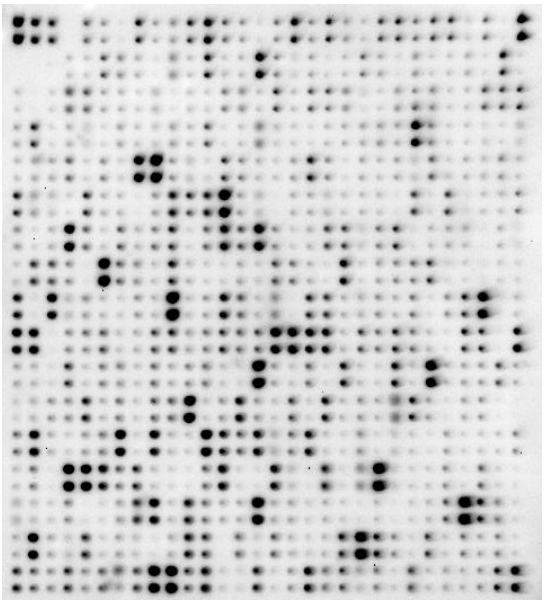
RayBio® L-Series Human Antibody Array L-3 List continued

number	name	number	name	number	name	number	name
301	DCI	361	ERAP2	421	GCSH	481	Histone H3.3
302	DCXR	362	ERp29	422	GDA	482	Histone H4
303	DDAH1	363	ERp57	423	GDF7	483	HLA-C
304	DDT	364	ERp72	424	GDI1	484	HMGB1
305	DDX3Y	365	ESD	425	GDI2	485	HMGB2
306	DEFA6	366	ESR1	426	Gephyrin	486	HMGB3
307	DEP-1	367	ETL	427	GFAP	487	HMGN2
308	Der p2	368	EVC2	428	GHRF	488	HN1
309	Dermcidin	369	Ezrin	429	GIP	489	HNF-3 alpha /FoxA1
310	Desmocollin 1	370	F11	430	GLIPR2	490	hnRNP A1
311	Desmocollin-2	371	FABP5	431	GLRX1	491	hnRNP A2B1
312	Desmocollin-3	372	Factor IX	432	G6PD	492	hnRNP C1 + C2
313	Desmoglein-1	373	Factor V	433	Glucosidase 2 subunit beta/PRKCSH	493	hnRNP G
314	Desmoglein-2	374	Factor XII	434	GLUD1	494	hnRNP L
315	Desmoplakin	375	Factor XIII	435	Glutamyl hydrolase gamma /CGH	495	hnRNP M1-M4
316	Desmuslin	376	FAM20C	436	GSTO1	496	hnRNP U
317	Destrin	377	FAM3C	437	Glutathione Synthetase/GSS	497	Hornerin
318	DGK	378	Fascin	438	Glycerol 3 Phosphate Dehydrogenase	498	Hoxb3
319	DISC 1	379	FASN	439	Glycoprotein V	499	HOXD11
320	DMGDH	380	fast skeletal Myosin	440	Glyoxalase II	500	HP1BP3
321	DMRN9	381	FASTKD5	441	GM2A	501	HPD
322	Dopamine beta Hydroxylase/DBH	382	FBP 38	442	GMF beta	502	HPR
323	DOT1L	383	FBP2	443	GNB1	503	HPRT
324	DPEP2	384	FBPase 1	444	GNPTG	504	HRG
325	DPP3	385	FCGBP	445	GOLPH2	505	HRSP12
326	DPPI	386	FDPS	446	GOLPH4	506	HSC70
327	DRIL1	387	FH	447	GOT2	507	HSP47
328	DSCAM	388	Fibrillin 1	448	GPCR GPR116	508	Pos 1b
329	DSPG3	389	Fibrinogen gamma chain/FGG	449	GPLD1	509	Pos 2b
330	Dystroglycan	390	Fibrinogen-like 2	450	Grainyhead-like protein 1 homolog/GRHL1	510	Pos 3b
331	E1 Ubiquitin Activating Enzyme/UBA1	391	Fibrinopeptide B	451	Granzyme M	511	Blank
332	ECHS1	392	Fibulin 3	452	GRHPR	512	Blank
333	ECM-1	393	Ficolin-2	453	GRP	513	Blank
334	EEF1G	394	Filamin A	454	GSTM1	514	Blank
335	EEF2	395	Filamin B	455	GSTP1	515	Blank
336	EFEMP2	396	Filamin C	456	Guanylin	516	Blank
337	EFTUD2	397	FKBP12	457	GULP1/CED-6	517	Blank
338	EHD1	398	FKBP25	458	H6PD	518	Blank
339	EHD3	399	FKBP51	459	HABP2	519	Blank
340	EIF3S2	400	FLG2	460	HBZ	520	Blank
341	eIF4A1-N-t	401	FOLR3	461	HCFC1	521	Blank
342	eIF5A	402	Frizzled 8	462	HDGF	522	Blank
343	ELAVL1	403	FRY	463	HEG1	523	Blank
344	EMILIN1	404	FSH	464	Hemoglobin	524	Blank
345	EMSY	405	FTL	465	Hemoglobin A1c	525	Blank
346	EN2	406	FUCA1	466	Hemoglobin subunit beta/HBB	526	Blank
347	Endorepellin	407	FUCA2	467	Hemoglobin subunit delta/HBD	527	Blank
348	ENO1	408	Fumarylacetoacetate hydrolase/FAH	468	Hemoglobin subunit gamma 2/HBG2	528	Blank
349	ENO1 + ENO2 + ENO3	409	G0/G1switch 2	469	HEXB	529	Blank
350	ENSA	410	G3BP	470	HGFA	530	Blank
351	Envoplakin	411	GALNT2	471	hGH	531	Blank
352	Eosinophil derived neurotoxin/EDN	412	gamma Catenin	472	hHR23b	532	Blank
353	EPB41	413	GAPDH	473	HIBADH	533	Blank
354	EPCR	414	GARNL1	474	HINT1	534	Blank
355	Ephrin B1	415	GART	475	HIP1R	535	Blank
356	Ephrin B2	416	Gastrokine 1	476	Histone H1.2	536	Blank
357	EPHX2	417	GATM - C-terminal	477	Histone H1.3	537	Blank
358	EPPK1	418	GBE1	478	Histone H2A	538	Blank
359	Eps 15	419	GCDFP 15	479	Histone H2A.Z	539	Blank
360	ERAB	420	GCLC	480	Histone H2B K	540	Blank

VI. Interpretation of Results

The following images show the RayBio® L-Series Human Antibody Array 1000 captured using a chemiluminescence imaging system (UVP BioImaging Systems). To obtain optimal results, 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. Anti-HRP (P-1a, P-2a, P-3a) and anti-streptavidin (P-1b, P-2b, P-3b) will produce positive control signals, which can be used to identify the orientation and help normalize the results from different arrays being compared.

L-3



Normal human sera was diluted 5x with 1X PBS and dialyzed against dialysis buffer before being labeled with biotin. Biotin-labeling, blocking and incubation of samples with the antibody array AAH-BLM-3 were performed according to the manual. The images were captured using a chemiluminescence imaging system (UVP BioImaging Systems).

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.

The RayBio® Analysis Tool is a program specifically designed for analysis of RayBio® L-Series Human Antibody Arrays. This tool will not only assist in compiling and organizing your data, but also reduces your calculations to a "copy and paste." Contact RayBiotech, Inc. at 770-729-2992 or info@raybiotech.com for ordering information.

VII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak signal or no signal	1. Taking too much time for detection.	1. The whole detection process must be completed in 30 min.
	2. Film developer does not work properly.	2. Fix film developer.
	3. Did not mix HRP-streptavidin well before use.	3. Mix tube containing HRP-Conjugate Streptavidin well before use since precipitates may form during storage.
	4. Sample is too dilute.	4. Increase sample concentration
	5. Other.	1.Check if there were any contamination with any solution containing amines in biotin-labeling step
		2. Slightly increase HRP concentrations.
		3. Work as quickly as possible after mix Detection Buffer C and D
		4. Expose film for overnight to detect weak signal.
Uneven signal	1. Bubbles formed during incubation.	1. Remove bubbles during incubation.
	2. Membranes were not completely covered by solution.	2. Completely cover membranes with solution.
High background	1. Exposure time is too long.	1. Decrease exposure time.
	2. Membranes dry out during experiment.	2. Completely cover membranes with solution during experiment. Cover tray w/ lid
	3. Sample is too concentrated.	3. Dilute sample.

VIII. Reference List

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