

# RayBio<sup>®</sup> Label-based (L-Series) Human Antibody Array 1000 Membrane Kit

A combination of Human L-507 and Human L-493 Arrays

Patent Pending Technology  
User Manual (Revised Feb 15, 2013)

For the simultaneous detection of the relative expression of 1000 human proteins in cell culture supernatants.

Human L-1000 Array (2 Sample Kit)  
Cat# AAH-BLM-1000-2

Human L-1000 Array (4 Sample Kit)  
Cat# AAH-BLM-1000-4

Please read manual carefully  
before starting experiment



Your Provider for Excellent Protein Array Systems and Services

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RayBiotech, Inc

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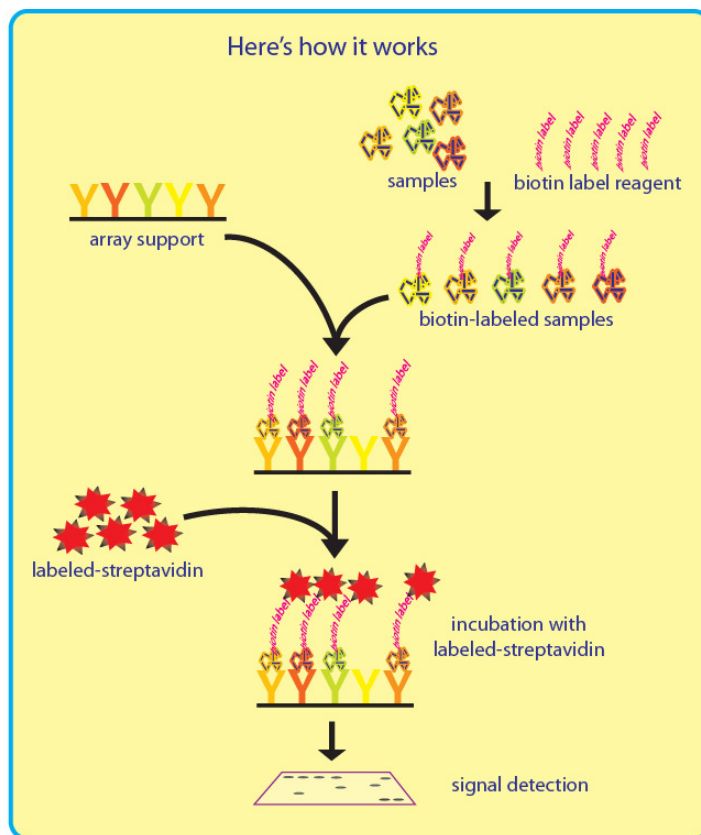
## TABLE OF CONTENTS

I.	Introduction.....	2
	How It Works.....	3
II.	Materials Provided.....	3
	A. Storage Recommendations.....	3
	B. Additional Materials Required.....	5
III.	Overview and General Considerations.....	5
	A. Handling Array Membranes.....	5
	B. Incubation of Antibody Array .....	5
IV.	Protocol.....	6
	A. Preparation of Samples.....	7
	B. Dialysis of Sample .....	8
	C. Biotin-labeling Sample .....	9
	D. Blocking and Incubation.....	11
	E. Detection.....	12
V.	Antibody Array Map.....	14
VI.	Interpretation of Results.....	20
VII.	Troubleshooting Guide.....	22
VIII.	Reference List.....	23

## I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the RayBio® L-Series Human Antibody Array 1000 (Human L-1000), researchers can now obtain a broad, panoramic view of cytokine expression. The expression levels of 1000 human proteins can be simultaneously detected, including cytokines, chemokines, adipokine, growth factors, angiogenic factors, proteases, soluble receptors, soluble adhesion molecules and other proteins in cell culture supernatants.

The first step in using the Human L-1000 is to biotinylate the primary amine 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 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 and Box 2 should be stored at  $-20\text{ }^{\circ}\text{C}$  and Box 3 and Box 4 should be stored at  $4\text{ }^{\circ}\text{C}$ . 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  $4\text{ }^{\circ}\text{C}$  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  $-20\text{ }^{\circ}\text{C}$  and avoid repeated freeze-thaw cycles (may be stored for up to 6 months).

## Box 1 and Box 2 (store at -20 °C):

		Box-1	Box 1 and Box-2
ITEM	DESCRIPTION	AAH-BLM-1000-2	AAH-BLM-1000-4
B	Labeling Reagent	2 vials	4 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio® L-Series Human 507 and 493 Antibody Array Membranes	4 membranes L-507 (2 membranes) L-493 (2 membranes)	8 membranes L-507 (4 membranes) L-493 (4 membranes)
F	Blocking Buffer	4 vials (30 ml/ea)	8 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 3 and Box 4 (store at 4 °C):

		Box-3	Box 3 and Box-4
ITEM	DESCRIPTION	AAH-BLM-1000-2	AAH-BLM-1000-4
A	Dialysis Vials	4 vials	8 vials
G	20X Wash Buffer 1 Concentrate	1 vial (30 ml)	2 vials (30 ml/ea)
H	20X Wash Buffer 2 Concentrate	1 vial (30 ml)	2 vials (30 ml/ea)
J	Spin Columns	4 columns	8 columns
N/A	Plastic Incubation Trays (w/lid)	4 trays	8 trays
M	Floating Dialysis Rack	1 rack	

## 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 tube

### **III. Overview and General Considerations**

#### **A. Handling Array Membranes**

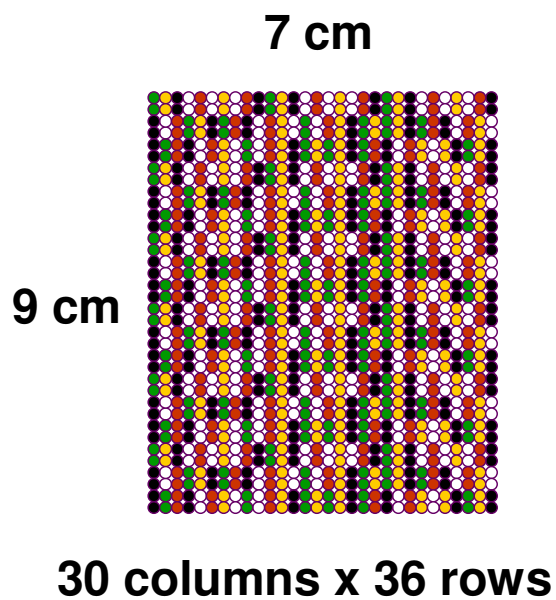
- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

#### **B. Incubation**

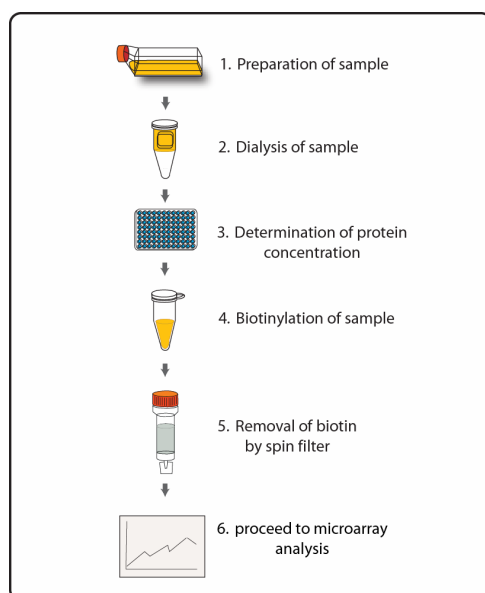
- Completely cover membranes with sample or buffer during incubation and cover Plastic Incubation Tray with lid to avoid drying.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Several incubation steps such as step 3 in page 10 (sample incubation) or step 7 in page 11 (HRP-Conjugated Streptavidin incubation) may be done at 4 °C for overnight.

## IV. Protocol

### Layout of L-507 and L-493 Array Membrane



### Assay Diagram



## **A. Preparation of Samples**

- 1). Seed cells at a density of  $1 \times 10^6$  cells in 100 mm tissue culture dishes.\*
- 2) Culture in complete culture medium for ~24–48 hours.\*\*
- 3) Replenish with serum-free or low-serum medium, such as 0.2% FCS/FBS, and then re-incubate cells for ~48 hours\*\*\*
- 4) Collect the cell culture supernatant and centrifuge at 1,000 g for 10 minutes and store in  $\leq 1$  ml aliquots at  $-80$  °C until needed.
- 5) Measure the total wet weight of the cultured cells in the pellet and/or culture dish. Normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Normalization can also be done between arrays by determining the total protein concentration using a total protein assay (RayBiotech recommends the Pierce BCA Protein Assay Kit, cat# 23227).

Note: \* The density of cells per dish used is dependent on the cell type. More or less cells may be required but should be determined empirically.

\*\* Optimal culture time may be different and depends on cell lines, treatment conditions, and other factors.

\*\*\* Bovine serum proteins produce detectable signals on the RayBio® L-Series membrane arrays at concentrations as low as 0.2%. When testing serum-containing media, it is



recommended test an uncultured media blank sample for comparison with sample results.

## **B. Dialysis of Sample**

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

1. Prepare dialysis buffer (1X PBS) by dissolving 0.6 g KCl, 24 g NaCl, 0.6 g  $\text{KH}_2\text{PO}_4$  and 3.45 g  $\text{Na}_2\text{HPO}_4$  in 2500 ml de-ionized or distilled water. Adjust to a pH of 8.0 with 1M NaOH and adjust final volume to 3000 ml with de-ionized or distilled water.
2. Load each sample into 2 separate Dialysis Vials (Item A), 2.5-3.0 ml of sample per vial, total 5-6 ml per sample) for dialyzing. Carefully place all Dialysis Vials into the Floating Rack.
3. Place the Floating Rack into  $\geq 500$  ml dialysis buffer in a large beaker. Place beaker on a stir plate and dialyze for at least 3 hours at 4 °C, occasionally gently stirring the dialysis buffer. Then exchange the dialysis buffer with fresh buffer and repeat dialysis for at least 3 hours at 4 °C. Transfer dialyzed samples into a clean eppendorf tube. 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.

### **C. Biotin-labeling of Sample**

Avoid contamination with any solution containing amines (i.e., Tris, glycine) as well as azides during the biotinylation process.

4. Immediately before use, prepare 1X Labeling Reagent by briefly centrifuging down the Labeling Reagent vial (Item B) and add 100  $\mu$ l 1X PBS (pH=8.0) into the vial. Pipette up and down or vortex briefly to dissolve the powder.
5. Add an appropriate amount\* of 1X Labeling Reagent into the tube containing the sample and immediately mix the reaction solution. Incubate the reaction solution at room temperature for 30 minutes with gentle shaking. Gently tap the tube to mix the reaction solution every 5 minutes.

\* Use 7.2  $\mu$ l of 1X Labeling Reagent for labeling 1 mg of total protein in samples. For example, if sample's total protein concentration is 0.5 mg/ml you need to add 10.8  $\mu$ l 1X Labeling Reagent to 3 ml dialyzed sample.

Note: The total protein concentration needs to be determined if the sample volume changes after dialysis or if the total protein concentration was determined before the dialysis step.

6. Add 5  $\mu$ l Stop Solution (Item D) into the reaction solution and then use the Spin Column (Item J) 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 50 ml conical collection tube.

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

Note: The resin should appear compacted after centrifugation.

c). Add 5 ml 1X PBS (pH=8.0) into the 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 50 ml conical collection tube and slowly load 3.5 ml of sample to the center of the compact resin bed.

Note: The maximal sample volume is 4 ml for each Spin Column. Do not load over 4 ml of 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 50 ml conical collection tube. Store at -80 <sup>0</sup>C until needed. Discard the Spin Column after use.

## **D. Blocking and Incubation**

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

Note: The printed membrane will have a “1” mark (for all L-507 membranes) “2” (for all L-493 membranes) in the upper left corner of the membrane.

8. Add 8 ml of Blocking Buffer (Item F) to each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour.
9. Aspirate Blocking Buffer from each tray. Add 8 ml of diluted\* or undiluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

Note: 1). It is recommended to use 8 ml of 5-fold diluted biotin-labeled cell culture supernatant. Dilute sample using Blocking Buffer.

Note: 2). The concentration of sample used depends on the abundance of 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: 3). Incubation may be done at room temperature with gentle shaking for 2 hours or overnight at 4°C.

10. Dilute 20X Wash Buffer 1 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 1 at room temperature with gentle shaking (5 min per wash). Repeat the wash 2 more times for a total of 3 washes.
11. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 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.
12. Aspirate the 1X Wash Buffer 2 from each tray. Dilute the 500X HRP-Conjugated Streptavidin with Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. 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.

13. Incubate at room temperature with gentle shaking for 2 hours.

Note: incubation may be done at 4 °C for overnight.

14. Wash as directed in steps 10 and 11.

## **E. Detection**

- \* Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.
15. 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 in 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 on to 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.
  16. 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.
  17. 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 exposure time (eg, 5–30 seconds). If the signals are too weak, increase exposure time (eg, 5–20 min or overnight). Or re-incubate membranes overnight with 1X HRP-Conjugated

Streptavidin, and repeat detection on the second day.

18. Save membranes at  $-20\text{ }^{\circ}\text{C}$  to  $-80\text{ }^{\circ}\text{C}$  for future reference.

## V. Antibody Array Map

RayBio® L-Series Human Antibody Array 1000 Maps – if needed, larger versions of these maps can be obtained by contacting technical support at 770-729-2992 or [techsupport@raybiotech.com](mailto:techsupport@raybiotech.com).

### RayBio® L-Series Human Antibody Array 507 (L-507) 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	
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	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	114	115	116	117	118	119	120	
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	
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B	B	B	B	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	
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B	B	B	B	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	
B	B	B	B	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	
B	B	B	B	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	
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B	B	B	B	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	N	N	N
B	B	B	B	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	N	N	N
B	B	B	B	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	N	P-3b	P-2b	P-1b	
B	B	B	B	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	N	P-3b	P-2b	P-1b	

# RayBio® L-Series Human Antibody Array 507 (L-507) List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	Positive 1a	61	CCR4	121	EN-RAGE	181	GDF-15	241	IL-1 F10 / IL-1HY2
2	Positive 2a	62	CCR5	122	Eotaxin / CCL11	182	GDNF	242	IL-1 R3 / IL-1 R AcP
3	Positive 3a	63	CCR6	123	Eotaxin-2 / MPIF-2	183	GFR alpha-1	243	IL-1 R4 /ST2
4	neg	64	CCR7	124	Eotaxin-3 / CCL26	184	GFR alpha-2	244	IL-1 R6 / IL-1 Rrp2
5	6Kine	65	CCR8	125	Epregrulin	185	GFR alpha-3	245	IL-1 R8
6	Activin A	66	CCR9	126	ErbB2	186	GFR alpha-4	246	IL-1 R9
7	Activin B	67	CD14	127	ErbB3	187	GITR / TNFRF18	247	IL-1 ra
8	Activin C	68	CD27 / TNFRSF7	128	ErbB4	188	GITR Ligand / TNFSF18	248	IL-1 sRI
9	Activin RIA / ALK-2	69	CD30 / TNFRSF8	129	Erythropoietin	189	Glucagon	249	IL-1 sRII
10	Activin RIB / ALK-4	70	CD30 Ligand / TNFSF8	130	E-Selectin	190	Glut1	250	IL-2
11	Activin RII A/B	71	CD40 / TNFRSF5	131	Endothelin	191	Glut2	251	IL-2 R alpha
12	Activin RIIA	72	CD40 Ligand / TNFSF5 / CD154	132	FADD	192	Glut3	252	IL-2 R beta / CD122
13	Adiponectin / Acrp30	73	CD 163	133	FAM3B	193	Glut5	253	IL-2 R gamma
14	AgRP	74	Cerberus 1	134	Fas / TNFRSF6	194	Glypican 3	254	IL-3
15	ALCAM	75	Chem R23	135	Fas Ligand	195	Glypican 5	255	IL-3 R alpha
16	Angiogenin	76	Chordin-Like 1	136	FGF Basic	196	GM-CSF	256	IL-4
17	Angiopoietin-1	77	Chordin-Like 2	137	FGF-BP	197	GM-CSF R alpha	257	IL-4 R
18	Angiopoietin-2	78	Csk	138	FGF R3	198	Granzyme A	258	IL-5
19	Angiopoietin-4	79	CLC	139	FGF R4	199	GREMLIN	259	IL-5 R alpha
20	Angiopoietin-like 1	80	CNTF	140	FGF R5	200	GRO	260	IL-6
21	Angiopoietin-like 2	81	CNTF R alpha	141	FGF-4	201	GRO-a	261	IL-6 R
22	Angiopoietin-like Factor	82	Coagulation Factor III / Tissue Factor	142	FGF-5	202	Growth Hormone (GH)	262	IL-7
23	Angiostatin	83	CRIM 1	143	FGF-6	203	Growth Hormone R (GHR)	263	IL-7 R alpha
24	APJ	84	Cripto-1	144	FGF-7 / KGF	204	HB-EGF	264	IL-8
25	AR (Amphiregulin)	85	CRTH-2	145	FGF-8	205	HCC-4 / CCL16	265	IL-9
26	APRIL	86	Cryptic	146	FGF-9	206	HCR / CRAM-A/B	266	IL-10
27	Artemin	87	CTACK / CCL27	147	FGF-10 / KGF-2	207	Hepassocin	267	IL-10 R alpha
28	Axl	88	CTGF / CCN2	148	FGF-11	208	Heregulin / NDF / GGF / Ne	268	IL-10 R beta
29	B7-1 / CD80	89	CTLA-4 / CD152	149	FGF-12	209	HGF	269	IL-11
30	BAFF R / TNFRSF13C	90	CV-2 / Crossveinless-2	150	FGF-13 1B	210	HGFR	270	IL-12 p40
31	neg	91	CXCL14 / BRAK	151	FGF-16	211	HRG1-alpha / NRG1-alpha	271	Blank
32	neg	92	CXCL16	152	FGF-17	212	HRG1-beta1 / NRG1-beta1	272	Blank
33	neg	93	CXCR1 / IL-8 RA	153	FGF-18	213	HVEM / TNFRSF14	273	Blank
34	BCMA / TNFRSF17	94	CXCR2 / IL-8 RB	154	FGF-19	214	I-309	274	Blank
35	BD-1	95	CXCR3	155	FGF-20	215	ICAM-1	275	IL-12 p70
36	BDNF	96	CXCR4 (fusin)	156	FGF-21	216	ICAM-2	276	IL-12 R beta 1
37	beta-Catenin	97	CXCR5 / BLR-1	157	FGF-23	217	ICAM-3 (CD50)	277	IL-12 R beta 2
38	beta-Defensin 2	98	CXCR6	158	FLRG	218	ICAM-5	278	IL-13
39	beta-NGF	99	D6	159	Flt-3 Ligand	219	IFN-alpha / beta R1	279	IL-13 R alpha 1
40	BIK	100	DAN	160	Follistatin	220	IFN-alpha / beta R2	280	IL-13 R alpha 2
41	BLC / BCA-1 / CXCL13	101	DANCE	161	Follistatin-like 1	221	IFN-beta	281	IL-15
42	BMP-2	102	DcR3 / TNFRSF6B	162	Fractalkine	222	IFN-gamma	282	IL-15 R alpha
43	BMP-3	103	Decorin	163	Frizzled-1	223	IFN-gamma R1	283	IL-16
44	BMP-3b / GDF-10	104	Dkk-1	164	Frizzled-3	224	IGFBP-1	284	IL-17
45	BMP-4	105	Dkk-3	165	Frizzled-4	225	IGFBP-2	285	IL-17B
46	BMP-5	106	Dkk-4	166	Frizzled-5	226	IGFBP-3	286	IL-17B R
47	BMP-6	107	DR3 / TNFRSF25	167	Frizzled-6	227	IGFBP-4	287	IL-17C
48	BMP-7	108	DR6 / TNFRSF21	168	Frizzled-7	228	IGFBP-6	288	IL-17D
49	BMP-8	109	Dtk	169	Galectin-3	229	IGFBP-rp1 / IGFBP-7	289	IL-17E
50	BMP-15	110	EDA-A2	170	GASP-1 / WFIKKNRP	230	IGF-I	290	IL-17F
51	BMPR-IA / ALK-3	111	EDAR	171	GASP-2 / WFIKKN	231	IGF-I SR	291	IL-17R
52	BMPR-IB / ALK-6	112	EDG-1	172	GCP-2 / CXCL6	232	IGF-II	292	IL-17RC
53	BMPR-II	113	EGF	173	GCSF	233	IGF-II R	293	IL-17RD
54	BTC	114	EGF R / ErbB1	174	G-CSF R / CD 114	234	IL-1 alpha	294	IL-18 BPa
55	Cardiotrophin-1 / CT-1	115	EG-VEGF / PK1	175	GDF1	235	IL-1 beta	295	IL-18 R alpha / IL-1 R5
56	CCL14 / HCC-1 / HCC-3	116	EMAP-II	176	GDF3	236	IL-1 F5 / FIL1delta	296	IL-18 R beta / AcPL
57	CCL28 / VIC	117	ENA-78	177	GDF5	237	IL-1 F6 / FIL1 epsilon	297	IL-19
58	CCR1	118	Endocan	178	GDF8	238	IL-1 F7 / FIL1 zeta	298	IL-20
59	CCR2	119	Endoglin / CD105	179	GDF9	239	IL-1 F8 / FIL1 eta	299	IL-20 R alpha
60	CCR3	120	Endostatin	180	GDF11	240	IL-1 F9 / IL-1 H1	300	IL-20 R beta



## RayBio® L-Series Human Antibody Array 507 (L-507) List continued

Number	Name	Number	Name	Number	Name	Number	Name
301	Blank	361	MFRP	421	PECAM-1 / CD31	481	Blank
302	Blank	362	MIF	422	Pentraxin3 / TSG-14	482	Blank
303	Blank	363	MIG	423	Persephin	483	Blank
304	Blank	364	MIP-1a	424	PF4 / CXCL4	484	Tie-1
305	IL-21	365	MIP-1b	425	PIGF	485	Tie-2
306	IL-21 R	366	MIP-1d	426	PLUNC	486	TIMP-1
307	IL-22	367	MIP 2	427	Pref-1	487	TIMP-2
308	IL-22 BP	368	MIP-3 alpha	428	Progranulin	488	TIMP-3
309	IL-22 R	369	MIP-3 beta	429	Prolactin	489	TIMP-4
310	IL-23	370	MMP-1	430	P-selectin	490	TL1A / TNFSF15
311	IL-23 R	371	MMP-2	431	RAGE	491	TLR1
312	IL-24	372	MMMP-3	432	RANK / TNFRSF11A	492	TLR2
313	IL-26	373	MMP-7	433	RANTES	493	TLR3
314	IL-27	374	MMP-8	434	RELM beta	494	TLR4
315	IL-28A	375	MMP-9	435	RELT / TNFRSF19L	495	TMEFF1 / Tomoregulin-1
316	IL-29	376	MMMP-10	436	ROBO4	496	TMEFF2
317	IL-31	377	MMP-11 / Stromelysin-3	437	S100 A8/A9	497	TNF-alpha
318	IL-31 RA	378	MMP-12	438	S100A10	498	TNF-beta
319	Inhibin A	379	MMP-13	439	SAA	499	TNF RI / TNFRSF1A
320	Inhibin B	380	MMP-14	440	SCF	500	TNF RII / TNFRSF1B
321	Insulin	381	MMP-15	441	SCF R / CD117	501	TRADD
322	Insulin R	382	MMP-16 / MT3-MMP	442	SDF-1 / CXCL12	502	TRAIL / TNFSF10
323	Insulysin / IDE	383	MMMP-19	443	sFRP-1	503	TRAIL R1 / DR4 / TNFRSF10A
324	IP-10	384	MMP-20	444	sFRP-3	504	TRAIL R2 / DR5 / TNFRSF10B
325	I-TAC / CXCL11	385	MMP-24 / MT5-MMP	445	sFRP-4	505	TRAIL R3 / TNFRSF10C
326	Kininostatin / kininogen	386	MMP-25 / MT6-MMP	446	sgp130	506	TRAIL R4 / TNFRSF10D
327	Kremen-1	387	Musk	447	SIGIRR	507	TRANCE
328	Kremen-2	388	MSP alpha Chain	448	Siglec-5/CD170	508	Neg
329	Lck	389	MICA	449	Siglec-9	509	Neg
330	Latent TGF-beta bp1	390	NAP-2	450	SLPI	510	Neg
331	Blank	391	NCAM-1 / CD56	451	Smad 1	511	Positive 2a
332	Blank	392	Neuritin	452	Smad 4	512	Positive 2b
333	Blank	393	NeuroD1	453	Smad 5	513	Positive 2c
334	Blank	394	Neuropilin-2	454	Smad 7	514	Blank
335	LBP	395	Neurturin	455	Smad 8	515	TREM-1
336	LECT2	396	NGF R	456	SMDF / NRG1 Isoform	516	TROY / TNFRSF19
337	Lefty - A	397	NOV / CCN3	457	Soggy-1	517	TSG-6
338	Leptin R	398	NRG1 Isoform GGF2	458	Sonic Hedgehog (Shh N-terminal)	518	TSLP R
339	Leptin (OB)	399	Nidogen-1	459	SPARC	519	TWEAK / TNFSF12
340	LFA-1 alpha	400	NrCam	460	Spinesin	520	TWEAK R / TNFRSF12
341	LIF	401	NRG2	461	TACI / TNFRSF13B	521	Ubiquitin+1
342	LIF R alpha	402	NRG3	462	Tarc	522	uPA
343	LIGHT / TNFSF14	403	NT-3	463	TCCR / WSX-1	523	uPAR
344	Lipocalin-1	404	NT-4	464	TECK / CCL25	524	Vasorin
345	LRP-1	405	Orexin A	465	TFPI	525	VCAM-1 (CD106)
346	LRP-6	406	Orexin B	466	TGF-alpha	526	VE-Cadherin
347	L-Selectin (CD62L)	407	OSM	467	TGF-beta 1	527	VEGF
348	Lipocalin-2	408	Osteoactivin / GPNMB	468	TGF-beta 2	528	VEGF R2 (KDR)
349	Lymphotactin / XCL1	409	Osteocrin	469	TGF-beta 3	529	VEGF R3
350	Lymphotoxin beta / TNFSF3	410	Osteoprotegerin / TNFRSF11B	470	TGF-beta 5	530	VEGF-B
351	Lymphotoxin beta R / TNFRSF3	411	OX40 Ligand / TNFSF4	471	TGF-beta RI / ALK-5	531	VEGF-C
352	MAC-1	412	PARC / CCL18	472	TGF-beta RII	532	VEGF-D
353	MCP-1	413	PD-ECGF	473	TGF-beta RIIB	533	VEGI / TNFSF15
354	MCP-2	414	PDGF R alpha	474	TGF-beta RIII	534	WIF-1
355	MCP-3	415	PDGF R beta	475	Thrombopoietin (TPO)	535	WISP-1 / CCN4
356	MCP-4 / CCL13	416	PDGF-AA	476	Thrombospondin (TSP)	536	XEDAR
357	M-CSF	417	PDGF-AB	477	Thrombospondin-1	537	Neg
358	M-CSF R	418	PDGF-BB	478	Thrombospondin-2	538	Positive 3b
359	MDC	419	PDGF-C	479	Thrombospondin-4	539	Positive 2b
360	MFG-E8	420	PDGF-D	480	Thymopoietin	540	Positive 1b

# RayBio® L-Series Human Antibody Array 493 (L-493) 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
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	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	114	115	116	117	118	119	120
91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
B	B	B	B	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
B	B	B	B	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
B	B	B	B	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
B	B	B	B	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
B	B	B	B	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360
B	B	B	B	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360
361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390
361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390
391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420
391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420
421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450
421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450
451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480
451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480
B	B	B	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	N	N	N
B	B	B	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	N	N	N
B	B	B	B	515	516	517	518	519	520	521	522	B	B	B	B	B	B	B	B	B	B	B	B	B	B	N	P-3b	P-2b	P-1b
B	B	B	B	515	516	517	518	519	520	521	522	B	B	B	B	B	B	B	B	B	B	B	B	B	N	P-3b	P-2b	P-1b	

## RayBio® L-Series Human Antibody Array 493 (L-493) List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	Positive 1a	61	ApoD	121	CD74	181	EphA2	241	GMNN
2	Positive 2a	62	ApoE	122	CD79 alpha	182	EphA3	242	GPBB
3	Positive 3a	63	ApoE3	123	CD90	183	EphA4	243	GPI
4	neg	64	ApoH	124	CD97	184	EphA5	244	GPR-39
5	11b-HSD1	65	ApoM	125	CD200	185	EphA6	245	GPX1
6	2B4	66	APP	126	CEA	186	EphA7	246	GPX3
7	4-1BB	67	ASPH	127	CEACAM-1	187	EphA8	247	GRP
8	A1BG	68	Attractin	128	Ceruloplasmin	188	EphB1	248	GRP75
9	A2M	69	B3GNT1	129	CFHR2	189	EphB2	249	GRP78
10	ABL1	70	BAF57	130	Chemerin	190	EphB3	250	GSR
11	ACE	71	BAFF	131	CHI3L1	191	EphB4	251	GST
12	ACE-2	72	BAI-1	132	Chromogranin A	192	EphB6	252	HADHA
13	ACK1	73	BCAM	133	Chymase	193	ERRa	253	HAI-1
14	ACPP	74	Beta 2M	134	clAP-2	194	Erythropoietin R	254	HAI-2
15	ACTH	75	Beta Defensin 4	135	Ck beta 8-1	195	ESAM	255	Haptoglobin
16	ADAM-9	76	Beta IG-H3	136	CK-MB	196	EV15L	256	hCG alpha
17	ADAMTS-1	77	Biglycan	137	Claudin-3	197	EXTL2	257	hCGb
18	ADAMTS-10	78	BLAME	138	Claudin-4	198	FABP1	258	Hck
19	ADAMTS-13	79	BMP-9	139	CLEC3B	199	FABP2	259	HE4
20	ADAMTS-15	80	BMX	140	Clusterin	200	FABP3	260	Hemopexin
21	ADAMTS-17	81	BNIP2	141	CNDP1	201	FABP4	261	Hepcidin
22	ADAMTS-18	82	BNP	142	COCO	202	Factor XIII A	262	HOXA10
23	ADAMTS-19	83	Btk	143	Complement factor H	203	Factor XIII B	263	HSP10
24	ADAMTS-4	84	C2	144	Contactin-1	204	FAK	264	HSP20
25	ADAMTS-5	85	C3a	145	Contactin-2	205	FAP	265	HSP27
26	ADAMTS-L2	86	C5/C5a	146	Corticosteroid-binding globulin	206	Fc RIIB/C	266	HSP32
27	Adipsin	87	C7	147	COX-2	207	Fen 1	267	HSP40
28	Afamin	88	C8B	148	C-peptide	208	FER	268	HSP60
29	AFP	89	C9	149	CNP2	209	Ferritin	269	HSP70
30	ALBUMIN	90	CA9	150	Creatinine	210	Fetuin A	270	HSP90
31	neg	91	CA15-3	151	CRP	211	Fetuin B	271	Blank
32	neg	92	CA19-9	152	CRTAM	212	FGFR1	272	Blank
33	neg	93	CA125	153	CSH1	213	FGFR1 alpha	273	Blank
34	Aldolase A	94	Cadherin-13	154	cTnT	214	FGFR2	274	Blank
35	Aldolase B	95	Calbindin	155	CutA	215	Fibrinogen	275	HSPA8
36	Aldolase C	96	Calbindin D	156	Cyclin D1	216	Fibrinopeptide A	276	HTRA2
37	ALK	97	Calcitonin	157	Cystatin A	217	Fibronectin	277	IBSP
38	Alpha 1 AG	98	Calreticulin	158	Cystatin B	218	Ficolin-3	278	IGF2BP1
39	Alpha 1 Microglobulin	99	Calsyntenin-1	159	Cystatin C	219	FIH	279	IGFBP-5
40	Alpha Lactalbumin	100	CART	160	Cytochrome C	220	FOLR1	280	IL-23p19
41	ALPP	101	Caspase-3	161	Cytokeratin 8	221	FOXP3	281	IL-33
42	AMICA	102	Caspase-8	162	Cytokeratin18	222	FoxO1	282	IL-34
43	AMPKa1	103	Cathepsin B	163	Cytokeratin19	223	FoxP3	283	IL36RN
44	Amylin	104	Cathepsin D	164	DBI	224	FRK	284	INSL3
45	ANGPTL3	105	Cathepsin L	165	DCBLD2	225	FSH	285	INSRR
46	ANGPTL4	106	Cathepsin S	166	D-Dimer	226	Furin	286	Integrin alpha V
47	Annexin A7	107	CBP	167	DEFA1/3	227	Fyn	287	Itk
48	APC	108	CCK	168	Defensin	228	GADD45A	288	ITM2B
49	APCS	109	CD23	169	Desmin	229	Galanin	289	Kallikrein 2
50	Apelin	110	CD24	170	DLL1	230	Galectin-1	290	Kallikrein 5
51	Apex1	111	CD36	171	DLL4	231	Galectin-3BP	291	Kallikrein 6
52	APN	112	CD38	172	DMP-1	232	Galectin-7	292	Kallikrein 7
53	ApoA1	113	CD44	173	DPPIV	233	gamma-Thrombin	293	Kallikrein 8
54	ApoA2	114	CD45	174	E-Cadherin	234	Gas1	294	Kallikrein 10
55	ApoA4	115	CD46	175	Endorphin Beta	235	Gastrin	295	Kallikrein 11
56	ApoB	116	CD47	176	Endothelin Receptor A	236	GATA-3	296	Kallikrein 14
57	ApoB100	117	CD55	177	Enolase 2	237	GATA-4	297	KCC3
58	ApoC1	118	CD59	178	ENPP2	238	Gelsolin	298	KCTD10
59	ApoC2	119	CD61	179	EpCAM	239	Ghrelin	299	KIF3B
60	ApoC3	120	CD71	180	EphA1	240	GLP-1	300	KLF4

# RayBio® L-Series Human Antibody Array 493 (L-493) List continued

Number	Name	Number	Name	Number	Name	Number	Name
301	Blank	361	Osteopontin	421	ROCK1	481	Blank
302	Blank	362	OX40	422	ROCK2	482	Blank
303	Blank	363	p21	423	ROR1	483	Blank
304	Blank	364	p27	424	ROR2	484	TRA-1-60
305	LAG-3	365	p53	425	ROS	485	TRA-1-81
306	Layilin	366	PAI-1	426	RYK	486	Transferrin
307	LDL R	367	PAK7	427	S100A4	487	Trappin-2
308	Legumain	368	Pancreastatin	428	S100A6	488	TRKB
309	LH	369	Pancreatic Polypeptide	429	S100A8	489	Troponin I
310	LIMP11	370	Pappalysin-1	430	S-100b	490	Troponin C
311	LIN41	371	PARK7	431	SART1	491	TRPC1
312	Livin	372	P-Cadherin	432	SART3	492	TRPC6
313	LOX-1	373	PCAF	433	SCG3	493	TRPM7
314	LPS	374	PD-1	434	Selenoprotein P	494	Trypsin 1
315	LRG1	375	PDX-1	435	SEMA3A	495	TSH
316	LTF	376	PDF	436	Serotonin	496	TSLP
317	LTK	377	PEPSINOGEN I	437	Serpin 1	497	TXK
318	Lumican	378	PEPSINOGEN II	438	Serpin A1	498	Tyk2
319	Lyn	379	PGRP-S	439	Serpin A3	499	TYRO10
320	LYRIC	380	PI 16	440	Serpin A4	500	Uromodulin
321	LYVE-1	381	PI 3Kinase p85 beta	441	Serpin A5	501	Vasopressin
322	LZTS1	382	PIM2	442	Serpin A8	502	VDUP-1
323	Mammaglobin A	383	PKM2	443	Serpin A9	503	VEGF R1
324	Marapsin	384	Plasminogen	444	Serpin A12	504	VEGF
325	MATK	385	Podocalyxin	445	Serpin B5	505	VIP Receptor 2
326	MBL	386	POMC	446	Serpin D1	506	Visfatin
327	MBL-2	387	PON1	447	Serpin I1	507	Vitamin D Receptor
328	Mer	388	PON2	448	SERTAD2	508	Neg
329	Mesothelin	389	PPARG2	449	SHBG	509	Neg
330	MICB	390	PPP2R5C	450	SMAC	510	Neg
331	Blank	391	Presenilin 1	451	SNCG	511	Blank
332	Blank	392	Presenilin 2	452	Somatotropin	512	Blank
333	Blank	393	Pro-BDNF	453	SOST	513	Blank
334	Blank	394	Procalcitonin	454	SOX17	514	Blank
335	Midkine	395	Pro-Cathepsin B	455	SOX2	515	Vitamin D-BP
336	MINA	396	Progesterone	456	SPARCL1	516	Vitamin K-dependent protein S
337	MSHa	397	pro-Glucagon	457	SPINK1	517	Vitronectin
338	MTUS1	398	Prohibitin	458	SRMS	518	VWF
339	Myoglobin	399	Pro-MMP-7	459	SSEA-1	519	Wilms Tumor 1
340	NAIP	400	Pro-MMP-9	460	SSEA-4	520	XIAP
341	Nanog	401	Pro-MMP-13	461	SSTR2	521	ZAG
342	NELL2	402	ProSAAS	462	SSTR5	522	ZAP70
343	NEP	403	Prostasin	463	Survivin	523	Blank
344	Nesfatin	404	Protein p65	464	SYK	524	Blank
345	Nestin	405	PSA-Free	465	Syndecan-1	525	Blank
346	NET1	406	PSA-total	466	Syndecan-3	526	Blank
347	Netrin G2	407	PSP	467	TACE	527	Blank
348	Netrin-4	408	PTH	468	TAF4	528	Blank
349	Neurokinin-A	409	PTHLP	469	Tec	529	Blank
350	Neuropeptide Y	410	PTN	470	TFF1	530	Blank
351	NF1	411	PTPRD	471	TFF3	531	Blank
352	NM23-H1/H2	412	PYK2	472	Thrombin	532	Blank
353	Notch-1	413	PYY	473	Thrombomodulin	533	Blank
354	NPTX1	414	Ras	474	Thymidine Kinase-1	534	Blank
355	NPTXR	415	RBP4	475	Thyroglobulin	535	Blank
356	NR3C3	416	RECK	476	TIM-1	536	Blank
357	Ntn1	417	RELM alpha	477	TNK1	537	Neg
358	OCT3/4	418	Resistin	478	TOPORS	538	Positive 3b
359	Omentin	419	RET	479	TPA	539	Positive 2b
360	Osteocalcin	420	RIP1	480	TPM1	540	Positive 1b

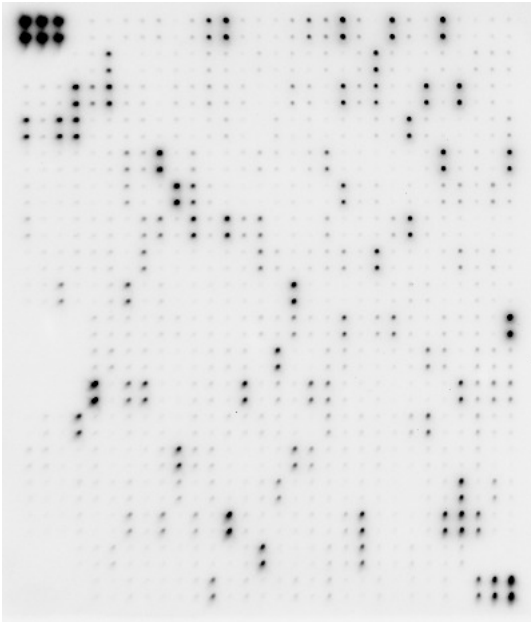
## VI. Interpretation of Results

The following images show the RayBio<sup>®</sup> 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.

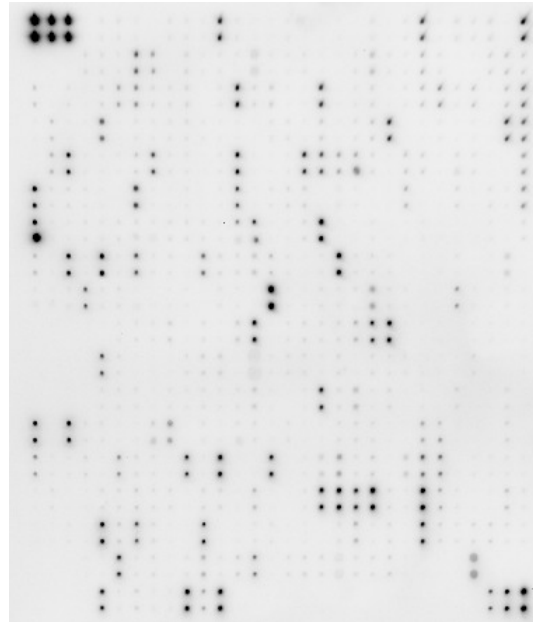
Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies.

The RayBio<sup>®</sup> Analysis Tool is a program specifically designed for analysis of RayBio<sup>®</sup> 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.” Call RayBiotech, Inc. at 770-729-2992 for ordering information.

L-507



L-493



## VII. Troubleshooting Guide

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
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.

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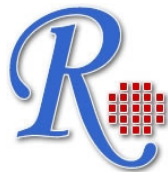
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