

# **RayBio<sup>®</sup> Label-Based (L-Series)**

## **Human Antibody Array L-2000 Membrane Kit**

A combination of Human L-507, L-493, L-3, and L-4 arrays

### **Patent Pending Technology**

### **User Manual (January 1, 2022)**

For the simultaneous detection of the relative expression of 2000 human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates and other body fluids.

**L-Series Human Antibody Array L-2000**  
**Cat# AAH-BLM-2000-2 (2 Sample Kit)**  
**Cat# AAH-BLM-2000-4 (4 Sample Kit)**

**Please read manual carefully  
before starting experiment**



**Your Provider of Excellent Protein Array Systems and Services**

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

## TABLE OF CONTENTS

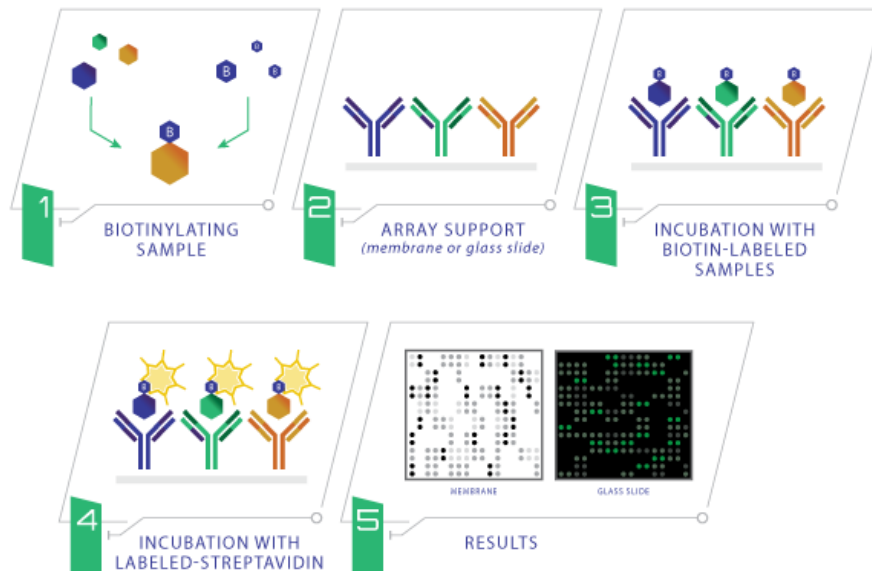
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I.	Introduction and How It Works.....	2
II.	Materials Provided.....	3
	A. Storage Recommendations.....	3
	B. Additional Materials Required.....	3
III.	Overview and General Considerations.....	4
	A. Preparation and Storage of Samples.....	4
	B. Handling the Array Membrane.....	6
	C. Incubation of Antibody Array.....	6
	D. Layout of Array Membrane.....	7
IV.	Protocol.....	8
	A. Sample Purification.....	8
	B. Biotin Labeling of Sample.....	9
	C. Blocking and Incubations.....	10
	D. Detection.....	12
V.	Antibody Array Map .....	13
VI.	Antibody Array Target Lists.....	15
VII.	Interpretation of Results.....	19
	A. Explanation of Controls Spots.....	19
	B. Typical Results.....	19
	C. Background Subtraction.....	20
	D. Normalization of Array Data.....	20
	E. Threshold of Significant Difference.....	21
	F. Pathway Analysis of the Array Proteins.....	21
VIII.	Troubleshooting Guide.....	22
IX.	Selected References.....	23

## 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 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, the kit should be stored at 4 °C or below and must be used within 6 months from the date of shipment. For longer period of storage, Labeling Reagent (Item B) and Array Membrane (Item E) should be stored at -20 °C and avoid repeated freeze-thaw cycles. Labeling Reagent (Item B) should be prepared fresh before use. After initial use, Labeling Buffer, 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).

ITEM	DESCRIPTION	2 SAMPLE KIT	4 SAMPLE KIT
B	Labeling Reagent	2 vials	4 vials
C	Labeling Buffer	1 bottle (30 ml)	1 bottle (30 ml)
D	Stop Solution	1 vial (50 µl)	1 vial (50 µl)
E	L-series Antibody Array Membranes	2 membranes each of Human L-507, L-493, L-3, and L-4	4 membranes each of Human L-507, L-493, L-3, and L-4
F	4X Blocking Buffer	2 bottles (30 ml)	3 bottles (30 ml)
I	500X HRP-Conjugated Streptavidin Concentrate	2 vials (100 µl)	3 vials (100 µl)
K	Detection Buffer C	2 bottles (10 ml)	4 bottles (10 ml)
L	Detection Buffer D	2 bottles (10 ml)	4 bottles (10 ml)
G	20X Wash Buffer 1 Concentrate	2 bottles (30 ml)	4 bottles (30 ml)
H	20X Wash Buffer 2 Concentrate	2 bottles (30 ml)	4 bottles (30 ml)
J-2	Spin Columns (10 ml)	4 columns	8 columns
N/A	Plastic Incubation Trays (w/lid)	8 trays	16 trays
N/A	2X Lysis Buffer	1 bottle (10 ml)	1 bottle (10 ml)
Other Kit Components: Plastic Sheets			

### B. Additional Materials Required

- 2-5 ml tube, small plastic or glass containers
- 50 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  $1 \times 10^6$  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 min and store as  $\leq 1$  ml aliquots at  $-80^\circ\text{C}$  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 the 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 min.

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<sub>2</sub>O). Solubilize the cells at  $2 \times 10^7$  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 min 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. Determine 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 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 ddH<sub>2</sub>O).

2. Homogenize the tissue according to homogenizer manufacturer 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 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**

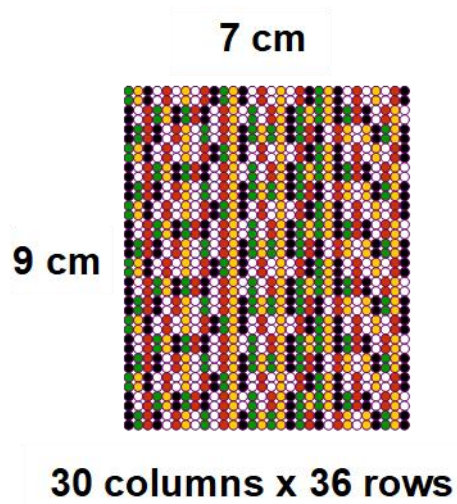
- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

### **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

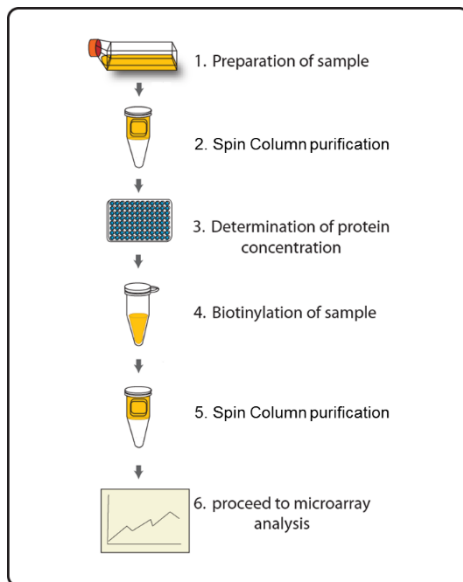




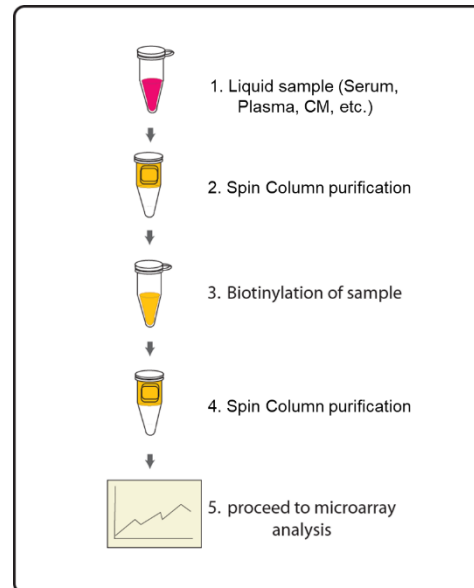
## IV. Protocol

### Assay Diagram

#### 1. Cell/tissue lysates



#### 2. Serum, plasma, body fluid, or Cell culture supernatants



### A. Sample purification

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

1. Twist to remove the bottom closure of the Spin column and loosen the cap (Do not remove).
2. Place the Spin column into a 50 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 5 ml ultrapure water (ddH<sub>2</sub>O) or 1xPBS (pH8.0) 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:

- *Culture Media (CM): 3.5 ml neat supernatant*
- *Serum/Plasma: 50 µl serum/plasma in 3.5 ml labeling buffer*
- *Cell/tissue lysate: 100 µg lysate in 1 ml labeling buffer*

*Note: Each labelled sample volume is enough for at least 6 membranes following the protocol below.*

*Note: The maximal sample volume is 4 ml for each Spin Column. Do not load over 4 ml 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 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 80 µl of Labeling Reagent into the sample tube (for 3.5 ml supernatant).
  - b. For labeling serum or plasma: Add 80 µl of Labeling Reagent Solution into the sample tube (for 50 µl serum/plasma *in 3.5 ml labeling buffer*).

- c. For labeling cell or tissue lysates: Add 8  $\mu$ l of Labeling Reagent Solution into the sample tube (for 100  $\mu$ g lysate *in 1 ml labeling buffer*).
- d. For all other body fluid: Add 2  $\mu$ l of Labeling Reagent Solution per 100  $\mu$ g 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  $\mu$ l Stop Solution (Item D) into each reaction 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 fluids. 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 1 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 Maps

### A. RayBio® Human Antibody Array L-507 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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## B. RayBio® Human Antibody Array L-493 Array Map

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11	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	
12	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	
13	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	
14	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	
15	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	
16	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	
17	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	
18	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	
19	Blank	Blank	Blank	Blank	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	
20	Blank	Blank	Blank	Blank	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	
21	Blank	Blank	Blank	Blank	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	
22	Blank	Blank	Blank	Blank	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	
23	Blank	Blank	Blank	Blank	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	
24	Blank	Blank	Blank	Blank	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	
25	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	
26	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	
27	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	
28	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	
29	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	
30	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	
31	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	
32	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	
33	Blank	Blank	Blank	Blank	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	Blank	Blank	
34	Blank	Blank	Blank	Blank	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	Blank	Blank	
35	Blank	Blank	Blank	Blank	486	487	488	489	490	491	492	493	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	
36	Blank	Blank	Blank	Blank	486	487	488	489	490	491	492	493	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	

## C. RayBio® Human Antibody Array L-3 and L-4 Array Map

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
1	POS1	POS2	POS3	Blank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
2	POS1	POS2	POS3	Blank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
3	Blank	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
4	Blank	Blank	Blank	Blank	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
5	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	
6	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	
7	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	
8	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	
9	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	
10	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	
11	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	
12	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	
13	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	
14	174	175	176																												

# VI. Antibody Array Target Lists

## A. RayBio® Human Antibody Array L-507 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	GCKine	74	CNTF R alpha	147	FGF-19	220	IGFBP-4	293	IL-22 BP	366	MMP-24	439	Shh-N
2	Activin A	75	F3	148	FGF-20	221	IGFBP-6	294	IL-22 R	367	MMP-25	440	SPARC
3	Activin B	76	CRIM 1	149	FGF-21	222	IGFBP-rp1	295	IL-23	368	Musk	441	Spinesin
4	Activin C	77	Cripto-1	150	FGF-23	223	IGF-I	296	IL-23 R	369	MSPa	442	TACI
5	Activin RIIA	78	CRTH-2	151	FLRG	224	IGF-I R	297	IL-24	370	MICA	443	Tarc
6	Activin RIB	79	Cryptic	152	Flt-3 Ligand	225	IGF-II	298	IL-26	371	NAP-2	444	TCCR
7	EYA2	80	CTACK	153	Follistatin	226	IGF-II R	299	IL-27	372	NCAM-1	445	TECK
8	Activin RIIA	81	CTGF	154	Follistatin-like 1	227	IL-1 alpha	300	IL-28A	373	Neuritin	446	TFPI
9	Adiponectin	82	CTLA-4	155	Fractalkine	228	IL-1 beta	301	IL-29	374	NeuroD1	447	TGF-alpha
10	AgRP	83	CV-2	156	Frizzled-1	229	IL-1 F5	302	IL-31	375	Neuropilin-2	448	TGF-beta 1
11	ALCAM	84	CXCL14	157	Frizzled-3	230	IL-1 F6	303	IL-31 RA	376	Neurturin	449	TGF-beta 2
12	Angiogenin	85	CXCL16	158	Frizzled-4	231	IL-1 F7	304	BACE-1	377	NGF R	450	TGF-beta 3
13	Angiopoietin-1	86	CXCR1	159	Frizzled-5	232	IL-1 F8	305	FACX	378	NOV	451	ATP2B1
14	Angiopoietin-2	87	CXCR2	160	Frizzled-6	233	IL-1 F9	306	Insulin	379	GGF2	452	TGF-beta RI
15	Angiopoietin-4	88	CXCR3	161	Frizzled-7	234	IL-1 F10	307	Insulin R	380	Nidogen-1	453	TGF-beta RII
16	ANGPTL1	89	CXCR4	162	Galectin-3	235	IL-1 R3	308	Insulysin	381	NrCam	454	Grb2
17	ANGPTL2	90	CXCR5	163	GASP-1	236	IL-1 R4	309	IP-10	382	NRG2	455	TGF-beta RIII
18	ANGPTL7	91	CXCR6	164	GASP-2	237	IL-1 R6	310	I-TAC	383	NRG3	456	Thrombopoietin
19	Angiostatin	92	D6	165	GCP-2	238	IL-1 R8	311	Kininostatin	384	NT-3	457	Thyroid Peroxidase
20	APJ	93	DAN	166	GCSF	239	IL-1 R9	312	Kremen-1	385	NT-4	458	Thrombospondin-1
21	Amphiregulin	94	DANCE	167	G-CSF R	240	IL-1 ra	313	Kremen-2	386	Orexin A	459	Thrombospondin-2
22	APRIL	95	DcR3	168	GDF1	241	IL-1 RI	314	Lck	387	Orexin B	460	Thrombospondin-4
23	Artemin	96	Decorin	169	GDF3	242	IL-1 RII	315	LTPB1	388	OSM	461	Thymopoietin
24	Axl	97	Dkk-1	170	GDF5	243	IL-2	316	LBP	389	Osteoactivin	462	Tie-1
25	B7-1	98	Dkk-3	171	GDF8	244	IL-2 R alpha	317	LECT2	390	Osteonin	463	Tie-2
26	BAFF R	99	Dkk-4	172	GDF9	245	IL-2 R beta	318	Lefty-A	391	Osteoprotegerin	464	TIMP-1
27	BCMA	100	DR3	173	GDF11	246	IL-2 R gamma	319	Leptin R	392	OX40 Ligand	465	TIMP-2
28	BD-1	101	DR6	174	GDF-15	247	IL-3	320	Leptin	393	PARC	466	TIMP-3
29	BDNF	102	Dtk	175	GDNF	248	IL-3 R alpha	321	LFA-1 alpha	394	PD-ECGF	467	TIMP-4
30	beta-Catenin	103	EDA-A2	176	GFR alpha-1	249	IL-4	322	LIF	395	PDGF R alpha	468	DEFAs
31	Bax	104	EDAR	177	GFR alpha-2	250	IL-4 R	323	LIF R alpha	396	PDGF R beta	469	TLR1
32	beta-NGF	105	EDG-1	178	GFR alpha-3	251	IL-5	324	LIGHT	397	PDGF-AA	470	TLR2
33	BIK	106	EGF	179	GFR alpha-4	252	IL-5 R alpha	325	Lipocalin-1	398	PDGF-AB	471	TLR3
34	BLC	107	EGF R	180	GITR	253	IL-6	326	LRP-1	399	PDGF-BB	472	TLR4
35	BMP-2	108	EG-VEGF	181	GITR Ligand	254	IL-6 R	327	LRP-6	400	PDGF-C	473	TMEFF1
36	BMP-3	109	EMAP-II	182	CBR1	255	IL-7	328	L-Selectin	401	PDGF-D	474	TMEFF2
37	BMP-3b	110	ENA-78	183	Glut1	256	IL-7 R alpha	329	Lipocalin-2	402	PECAM-1	475	TNF-alpha
38	BMP-4	111	Endocan	184	Glut2	257	IL-8	330	Lymphotoctin	403	Pentraxin3	476	TNF-beta
39	BMP-5	112	Endoglin	185	Glut3	258	IL-9	331	LTB	404	Persephin	477	TNF RI
40	BMP-6	113	Endostatin	186	Glut5	259	IL-10	332	LTBR	405	PF4	478	TNF RII
41	BMP-7	114	EN-RAGE	187	Glypican 3	260	IL-10 R alpha	333	MAC-1	406	PIGF	479	TRADD
42	BMP-8	115	Eotaxin	188	Glypican 5	261	IL-10 R beta	334	MCP-1	407	PLUNC	480	TRAIL
43	BMP-15	116	Eotaxin-2	189	GM-CSF	262	IL-11	335	MCP-2	408	Pref-1	481	TRAIL R1
44	BMPI-IA	117	Eotaxin-3	190	GM-CSF R alpha	263	IL-12 p40	336	MCP-3	409	Progranulin	482	TRAIL R2
45	BMPI-IB	118	Epregrulin	191	Granzyme A	264	IL-12 p70	337	MCP-4	410	Prolactin	483	TRAIL R3
46	BMPI-II	119	ErbB2	192	GREMLIN	265	IL-12 R beta 1	338	M-CSF	411	P-selectin	484	TRAIL R4
47	BTC	120	ErbB3	193	GRO	266	IL-12 R beta 2	339	M-CSF R	412	RAGE	485	TRANCE
48	Cardiotrophin-1	121	ErbB4	194	GRO-a	267	IL-13	340	MDC	413	RANK	486	TREM-1
49	CCL14	122	Erythropoietin	195	GH	268	IL-13 R alpha 1	341	MFG-E8	414	RANTES	487	TROY
50	CCL28	123	E-Selectin	196	GHR	269	IL-13 R alpha 2	342	MFRP	415	RELM beta	488	TSG-6
51	CCR1	124	Endothelin	197	HB-EGF	270	IL-15	343	MIF	416	RELT	489	TSLP R
52	CCR2	125	FADD	198	HCC-4	271	IL-15 R alpha	344	MIG	417	ROBO4	490	TWEAK
53	CCR3	126	FAM3B	199	HCR	272	IL-16	345	MIP-1a	418	S100 A8/A9	491	TWEAK R
54	CCR4	127	Fas	200	Hepassocin	273	IL-17	346	MIP-1b	419	S100A10	492	Ubiquitin+1
55	CCR5	128	Fas Ligand	201	GLO-1	274	IL-17B	347	MIP-1d	420	SAA	493	uPA
56	CCR6	129	FGF Basic	202	HGF	275	IL-17B R	348	MIP 2	421	SCF	494	uPAR
57	CCR7	130	FGF-BP	203	HGFR	276	IL-17C	349	MIP-3 alpha	422	SCF R	495	Vasorin
58	CCR8	131	FGF R3	204	HRG-alpha	277	IL-17D	350	MIP-3 beta	423	SDF-1	496	VCAM-1
59	CCR9	132	FGF R4	205	HRG-beta 1	278	IL-17E	351	MMP-1	424	sFRP-1	497	VE-Cadherin
60	CD14	133	FGF R5	206	HVEM	279	IL-17F	352	MMP-2	425	sFRP-3	498	VEGF
61	CD27	134	FGF-4	207	I-309	280	IL-17R	353	MMP-3	426	sFRP-4	499	VEGF R2
62	CD30	135	FGF-5	208	ICAM-1	281	IL-17RC	354	MMP-7	427	sgp130	500	VEGF R3
63	CD30 Ligand	136	FGF-6	209	ICAM-2	282	IL-17RD	355	MMP-8	428	SIGIRR	501	VEGF-B
64	CD40	137	FGF-7	210	ICAM-3	283	IL-18 Bpa	356	MMP-9	429	Siglec-5	502	VEGF-C
65	CD40 Ligand	138	FGF-8	211	ICAM-5	284	IL-18 R alpha	357	MMP-10	430	Siglec-9	503	VEGF-D
66	CD 163	139	FGF-9	212	IFN-alpha/beta R1	285	IL-18 R beta	358	MMP-11	431	SLPI	504	VEGI
67	Cerberus 1	140	FGF-10	213	IFN-alpha/beta R2	286	IL-19	359	MMP-12	432	Smad 1	505	WIF-1
68	Chem R23	141	FGF-11	214	IFN-beta	287	IL-20	360	MMP-13	433	Smad 4	506	WISP-1
69	Chordin-Like 1	142	FGF-12	215	IFN-gamma	288	IL-20 R alpha	361	MMP-14	434	Smad 5	507	XEDAR
70	Chordin-Like 2	143	FGF-13 1B	216	IFN-gamma R1	289	IL-20 R beta	362	MMP-15	435	Smad 7		
71	Csk	144	FGF-16	217	IGFBP-1	290	IL-21	363	MMP-16	436	Smad 8		
72	CLC	145	FGF-17	218	IGFBP-2	291	IL-21 R	364	MMP-19	437	Prdx6		
73	CNTF	146	FGF-18	219	IGFBP-3	292	IL-22	365	MMP-20	438	Soggy-1		



## B. RayBio® Human Antibody Array L-493 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	11b-HSD1	73	BMX	145	CRTAM	217	FRK	289	KLF4	361	PI 16	433	SCGF
2	2B4	74	BNIP2	146	CSH1	218	ARB1	290	LAG-3	362	PIK3R1	434	SOST
3	4-1BB	75	BNP	147	Troponin T	219	Furin	291	Layilin	363	PIM2	435	SOX17
4	A1BG	76	Btk	148	CutA	220	Fyn	292	LDL R	364	PKM2	436	SOX2
5	A2M	77	C2	149	Cyclin D1	221	GADD45A	293	Legumain	365	Plasminogen	437	SPARCL1
6	ABL1	78	C3a	150	Cystatin A	222	Galanin	294	LH	366	Podocalyxin	438	SPINK1
7	ACE	79	C5a	151	Cystatin B	223	Galectin-1	295	LIMPII	367	POMC	439	SRMS
8	ACE-2	80	C7	152	Cystatin C	224	Galectin-3BP	296	LN41	368	PON1	440	SSEA-1
9	ACK1	81	C8b	153	Cytochrome C	225	Galectin-7	297	Livin	369	PON2	441	SSEA-4
10	ACPP	82	C9	154	Cytokeratin 8	226	gamma-Thrombin	298	LOX-1	370	PPARg2	442	SSTR2
11	ACTH	83	CA9	155	Cytokeratin 18	227	Gas1	299	LPS	371	PPP2R3C	443	SSTR5
12	ADAM-9	84	CA15-3	156	Cytokeratin 19	228	Gastrin	300	LRG1	372	Presenilin 1	444	Survivin
13	ADAMTS1	85	CA19-9	157	DBI	229	GATA-3	301	LTF	373	Presenilin 2	445	SYK
14	ADAMTS10	86	CA125	158	DCBLD2	230	GATA-4	302	LTK	374	Pro-BDNF	446	Syndecan-1
15	ADAMTS13	87	Cadherin-13	159	D-Dimer	231	Gelsolin	303	Lumican	375	Procalcitonin	447	Syndecan-3
16	ADAMTS15	88	CLEC14A	160	DEFA1/3	232	Ghrelin	304	Lyn	376	Pro-Cathepsin B	448	TACE
17	ADAMTS17	89	Calbindin D	161	CPA1	233	GLP-1	305	LYRIC	377	Progesterone	449	TAF4
18	ADAMTS18	90	Calcitonin	162	Desmin	234	GMN	306	LYVE-1	378	pro-Glucagon	450	Tec
19	ADAMTS19	91	Calreticulin	163	DLL1	235	GPBB	307	LZTS1	379	Prohibitin	451	TFF1
20	ADAMTS4	92	Calsyntenin-1	164	DLL4	236	GPI	308	Mammaglobin A	380	Pro-MMP-7	452	TFF3
21	ADAMTS5	93	CART	165	DMP-1	237	GPR-39	309	Marapsin	381	Pro-MMP-9	453	Thrombin
22	ADAMTS2	94	Caspase-3	166	DPPIV	238	GPX1	310	MATK	382	Pro-MMP-13	454	Thrombospondin
23	Adipsin	95	Caspase-8	167	E-Cadherin	239	GPX3	311	MBL	383	ProSAAS	455	TK1
24	Afamin	96	Cathepsin B	168	Endorphin Beta	240	GRP	312	C1qTNF1	384	Prostasin	456	Thyroglobulin
25	AFP	97	Cathepsin D	169	EDNRA	241	GRP75	313	Mer	385	Protein p65	457	TIM-1
26	ALBUMIN	98	Cathepsin L	170	Enolase 2	242	GRP78	314	Mesothelin	386	PSA-Free	458	TNK1
27	Aldolase A	99	Cathepsin S	171	ENPP2	243	GSR	315	MICB	387	PSA-total	459	TOPORS
28	Aldolase B	100	CBP	172	EpCAM	244	GST	316	Midkine	388	PSP	460	TPA
29	Aldolase C	101	CCK	173	EphA1	245	HADHA	317	MINA	389	PTH	461	TPM1
30	ALK	102	CD23	174	EphA2	246	HAI-1	318	MShA	390	PTHLP	462	TRA-1-60
31	Alpha 1 AG	103	CD24	175	EphA3	247	HAI-2	319	MTUS1	391	PTN	463	TRA-1-81
32	A1M	104	CD36	176	EphA4	248	Haptoglobin	320	Myoglobin	392	PTPRD	464	Transferrin
33	Alpha Lactalbumin	105	CD38	177	EphA5	249	hCG alpha	321	NAIP	393	PYK2	465	Trappin-2
34	ALPP	106	CD44	178	EphA6	250	hCGb	322	Nanog	394	PYY	466	TRKB
35	AMICA	107	CD45	179	EphA7	251	Hck	323	NELL2	395	Ras	467	Troponin I
36	AMPKa1	108	CD46	180	EphA8	252	HE4	324	Nephrilysin	396	RBP4	468	Troponin C
37	Amylin	109	CD47	181	EphB1	253	Hemopexin	325	Nesfatin	397	RECK	469	TRPC1
38	ANGPTL3	110	CD55	182	EphB2	254	Hepcidin	326	Nestin	398	RELN alpha	470	TRPC6
39	ANGPTL4	111	CD59	183	EphB3	255	HOXA10	327	NET1	399	Resistin	471	TRPM7
40	Annexin A7	112	CD61	184	EphB4	256	HSP10	328	Netrin G2	400	RET	472	Trypsin 1
41	APC	113	CD71	185	EphB6	257	HSP20	329	Netrin-4	401	RIP1	473	TSH
42	APCS	114	CD74	186	ERRa	258	HSP27	330	Neurokinin A	402	ROCK1	474	TSLP
43	Apelin	115	CD79 alpha	187	Erythropoietin R	259	HSP32	331	Neuropeptide Y	403	ROCK2	475	TXK
44	Apex1	116	CD90	188	ESAM	260	HSPA0	332	NF1	404	ROR1	476	Tyk2
45	APN	117	CD97	189	EV15L	261	HSP60	333	NM23-H1/H2	405	ROR2	477	TYRO10
46	ApoA1	118	CD200	190	EXTL2	262	HSP70	334	Notch-1	406	ROS	478	Uromodulin
47	ApoA2	119	CEA	191	FABP1	263	HSP90	335	NPTX1	407	RYK	479	Vasopressin
48	ApoA4	120	CEACAM-1	192	FABP2	264	HSPA8	336	NPTXR	408	S100A4	480	VDUP-1
49	ApoB	121	Ceruloplasmin	193	FABP3	265	HTRA2	337	NR3C3	409	S100A6	481	VEGF R1
50	ApoB100	122	CFHR2	194	FABP4	266	IBSP	338	Ntn1	410	S100A8	482	VEGF
51	ApoC1	123	Chemerin	195	Fc gamma RIIB	267	IGF2BP1	339	OCT3/4	411	S-100b	483	VIPR2
52	ApoC2	124	CHI3L1	196	Factor XIII B	268	IGFBP-5	340	Omentin	412	SART1	484	Visfatin
53	ApoC3	125	Chromogranin A	197	FAK	269	IDUA	341	Osteocalcin	413	SART3	485	VDR
54	ApoD	126	Chymase	198	FAP	270	IL-33	342	Osteopontin	414	SCG3	486	VDB
55	ApoE	127	ciAP-2	199	Fcg RIIB/C	271	IL-34	343	OX40	415	Selenoprotein P	487	PROS1
56	ApoE3	128	Ck beta 8-1	200	Fen-1	272	IL-28B	344	p21	416	SEMA3A	488	Vitronectin
57	ApoH	129	CKMB	201	FER	273	INSL3	345	p27	417	Serotonin	489	VWF
58	ApoM	130	Claudin-3	202	Ferritin	274	INSRR	346	p53	418	Serpin G1	490	WT1
59	APP	131	Claudin-4	203	Fetuin A	275	ITGAV	347	PAI-1	419	Serpin A1	491	XIAP
60	ASPH	132	CLEC3B	204	Fetuin B	276	Itk	348	PAK7	420	Serpin A3	492	ZAG
61	Attractin	133	Clusterin	205	FGFR1	277	ITM2B	349	Pancreastatin	421	Serpin A4	493	ZAP70
62	B3GNT1	134	CNDP1	206	FGFR1 alpha	278	Kallikrein 2	350	PP	422	Serpin A5		
63	BAF57	135	COCO	207	FGFR2	279	Kallikrein 5	351	Pappalysin-1	423	Serpin A8		
64	BAFF	136	CFH	208	Fibrinogen	280	Kallikrein 6	352	PARK7	424	Serpin A9		
65	BAI-1	137	Contactin-1	209	Fibrinogen A	281	Kallikrein 7	353	P-Cadherin	425	Serpin A12		
66	BCAM	138	Contactin-2	210	Fibronectin	282	Kallikrein 8	354	PCAF	426	Serpin B5		
67	B2M	139	CBG	211	Ficolin-3	283	Kallikrein 10	355	PD-1	427	Serpin D1		
68	Beta Defensin 4	140	COX-2	212	FIH	284	Kallikrein 11	356	PDX-1	428	Serpin I1		
69	Beta IG-H3	141	C-peptide	213	FOLR1	285	Kallikrein 14	357	PDFF	429	SERTAD2		
70	Biglycan	142	CPN2	214	FOXN3	286	KCC3	358	PEPSINOGEN I	430	SHBG		
71	BLAME	143	Creatinine	215	FoxO1	287	KCTD10	359	PEPSINOGEN II	431	SMAC		
72	BMP-9	144	CRP	216	FoxP3	288	KIF3B	360	PGRP-S	432	SNCG		

# C. RayBio® Human Antibody Array L-3 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 beta	73	Antithrombin III	145	C4BPA	217	CHREBP	289	Cytokeratin 9	361	EVC2	433	Glyoxalase II
2	14-3-3 epsilon	74	APA	146	C5b-9	218	Chromogranin B	290	D4 GDI	362	Ezrin	434	GM2A
3	14-3-3 eta	75	APLP-1	147	C6	219	Chromogranin C	291	DAK	363	F11	435	GMF beta
4	14-3-3 gamma	76	APM2	148	C8G	220	CIP29	292	Contactin-4	364	FABP5	436	GNB1
5	14-3-3 sigma	77	Apo (a)	149	C9orf40	221	CKB	293	DARS2	365	Factor IX	437	GNPTG
6	14-3-3 theta	78	APOA1BP	150	CA1	222	CLIC1	294	DCI	366	Factor V	438	GOLPH2
7	14-3-3 zeta	79	ApoF	151	CA150	223	CLIC4	295	DCXR	367	Factor XII	439	GOLPH4
8	53BP1	80	ApoL1	152	CA2	224	CLIP170	296	DDAH1	368	Factor XIII	440	GOT2
9	67LR	81	ApoL2	153	CA3	225	CL-P1	297	DDT	369	FAM20C	441	GPR116
10	ABAT	82	ARFBP1	154	CACNB4	226	CLPS	298	DDX3Y	370	FAM3C	442	GPLD1
11	ABCF1	83	ARFGEF3	155	CAD	227	CLTA	299	DEFA6	371	Fascin	443	GRHL1
12	ABI3BP	84	ASL	156	Cadherin 22	228	CNN2	300	DEP-1	372	FASN	444	Granzyme M
13	ACAA1	85	ArgRS	157	Cadherin-6	229	CNOT1	301	DNER	373	fast skeletal Myosin	445	GRHRP
14	ACAA2	86	ARP19	158	CALD1	230	CO4A2	302	Dermcidin	374	FASTKD5	446	GRP
15	ACACA	87	Arp2	159	CALML5	231	COG4	303	Desmocollin 1	375	FBP38	447	GSTM1
16	ACAA	88	ARP2/3	160	Calmodulin	232	COL19A1	304	Desmocollin-2	376	FBP2	448	GSTP1
17	ACLP	89	Arp3	161	Calpain 1	233	COL4A3	305	Desmocollin-3	377	FBPase 1	449	Guanlylin
18	ACLY	90	ARPC2	162	Calpain S1	234	Col6A2	306	Desmoglein-1	378	FCGBP	450	GULP1
19	Aconitase 1	91	ARPC3	163	Calpastatin	235	COL9A3	307	Desmoglein-2	379	FDP5	451	H6PD
20	ACTBL2	92	ART3	164	Calretinin	236	COLEC10	308	Desmoplakin	380	FH	452	HABP2
21	ACTC1	93	ARTS1	165	Calumenin	237	Collagen I a1	309	Desmuslin	381	Fibrillin 1	453	HBZ
22	Actinin alpha 1	94	ARX	166	CAP1	238	Collagen III	310	Destrin	382	FGG	454	HCFC1
23	ADAMDEC1	95	ASH2L	167	CapG	239	Collagen IVa6	311	DGK	383	Fibrinogen-like 2	455	HDGF
24	ADAS	96	ASGR2	168	CAP2A1	240	Collagen IX	312	DISC 1	384	Fibrinopeptide B	456	HEG1
25	ADH1B	97	ASK1	169	CPB2	241	Collagen V	313	DMGDH	385	Fibulin 3	457	Hemoglobin
26	ADH1C	98	AST	170	CARHSP1	242	Collagen VI	314	DMRN9	386	Ficolin-2	458	Hemoglobin A1c
27	ADH4	99	DNPEP	171	Caspase-14	243	Collagen X	315	DBH	387	Filamin A	459	HBB
28	ADH5	100	ASXL1	172	Catalase	244	COL15A1	316	DOT1L	388	Filamin B	460	HBD
29	ADM	101	ATBF1	173	Cathelicidin	245	COMP	317	DPEP2	389	Filamin C	461	HBG2
30	Advillin	102	ATP5A	174	Cathepsin A	246	CFB	318	DPP3	390	FKBP12	462	HEXB
31	AFG3L2	103	ATP5O	175	Cathepsin G	247	Contactin-3	319	DPPI	391	FKBP25	463	HGFA
32	AGA	104	ATPB	176	Cathepsin H	248	COPE58	320	DRIL1	392	FKBP51	464	hGH
33	Aggrecan	105	B3GNT2	177	Cathepsin Z	249	Corneodesmosin	321	DSCAM	393	FLG2	465	hHR23b
34	AGXT	106	B4GalT1	178	CBS	250	Coronin 3	322	DSPG3	394	FOLR3	466	HIBADH
35	AHNAK	107	B7-H2	179	CCDC126	251	Cortactin	323	Dystroglycan	395	Frizzled 8	467	HINT1
36	Ahsp	108	B7-H3	180	CDC25	252	COTL1	324	UBA1	396	FRY	468	HIP1R
37	AIF	109	BAD	181	CCT3	253	CPE	325	ECHS1	397	FSH	469	Histone H1.2
38	AK2	110	Band 3	182	CD109	254	CPEB3	326	ECM-1	398	Azurocidin	470	Histone H1.3
39	AKAP9	111	BASP1	183	CD133	255	CPM	327	EEF1G	399	FUCA1	471	Histone H2A
40	AKR1B1	112	Bassoon	184	CD155	256	CPN1	328	EEF2	400	FUCA2	472	Histone H2A.Z
41	AKR1C3	113	BAZ2B	185	CD157	257	CPNE3	329	EFEMP2	401	FAH	473	Histone H2B K
42	AKR7A2	114	BCHE	186	CD16	258	CPS1	330	EFTUD2	402	GO/G1switch 2	474	Histone H3.3
43	ALAD	115	Bcl-w	187	CD21	259	CKMM	331	EHD1	403	G3BP	475	Histone H4
44	ALT	116	BCOR	188	CD32	260	CRF21	332	EHD3	404	GALNT2	476	HLC-C
45	ADH	117	beta 1 Spectrin	189	CD35	261	CRHBP	333	EIF3S2	405	gamma Catenin	477	HMGb1
46	AOX1	118	CRYBB1	190	CD39L4	262	CrkL	334	eIF4A1	406	GAPDH	478	HMGb2
47	ALDH16A1	119	beta 1 Tubulin	191	CD41	263	CRMP2	335	eIF5A	407	GARNL1	479	HMGb3
48	ALDH1A1	120	CUBB3	192	CD42b	264	CRTAC1	336	ELAVL1	408	GART	480	HMGn2
49	ALDH9A1	121	BID	193	CD48	265	CS	337	EMIUN1	409	Gastrokine 1	481	HN1
50	ALPK	122	BIN2	194	CD5L	266	Ctip2	338	EMSY	410	GATM	482	FoxA1
51	ALP	123	BIRC6	195	CD9	267	Cux2	339	EN2	411	GBE1	483	hnRNP A1
52	MAN1A1	124	BLMH	196	CD98	268	Cyclophilin A	340	Endorepellin	412	GCDPF 15	484	hnRNP A2B1
53	alpha Actinin 4	125	BLVRB	197	CDA	269	Cyclophilin B	341	ENO1	413	GCLC	485	hnRNP C1+C2
54	Alpha Fodrin	126	BMP-1	198	CDC5L	270	Cystatin D	342	ENO1+ENO2+ENO3	414	GCSH	486	hnRNP G
55	alpha Glucosidase II	127	BPGM	199	CDK2	271	Cystatin E	343	ENSA	415	GDA	487	hnRNP L
56	alpha-Synuclein	128	BPIFB1	200	CEACAM-8	272	Cystatin S	344	Envoplakin	416	GDF7	488	hnRNP M1-M4
57	alpha Tubulin	129	BPI1L	201	CECR1	273	Cystatin SN	345	EDN	417	GDI1	489	hnRNP U
58	CRYAA	130	BRCA 2	202	CENPF	274	CSRP1	346	EPB41	418	GDI2	490	Hornerin
59	ALS	131	BRD2	203	CEP57	275	CYTL1	347	EPCR	419	Gephyrin	491	Hoxb3
60	Als2	132	Brevican	204	CES1	276	Cytochrome b5	348	Ephrin B1	420	GFAP	492	HOXD11
61	ALS2CR1	133	Brg1	205	CETP	277	Cytochrome c (m)	349	Ephrin B2	421	GHRF	493	HP1BP3
62	Aminoacylase	134	BRSK1	206	Cezanne	278	Cytokeratin 1	350	EPHX2	422	GIP	494	HPD
63	Androgen Receptor	135	BDT	207	CFHR1	279	Cytokeratin 10	351	EPPK1	423	GLIPR2	495	HPR
64	ANGPTL6	136	BTf3	208	CFHR4	280	Cytokeratin 13	352	Eps15	424	GLRX1	496	HPRT
65	ANGPTL8	137	C1q	209	CFHR5	281	Cytokeratin 14	353	ERAB	425	G6PD	497	HRG
66	ANK	138	C1qA	210	CFI	282	Cytokeratin 15	354	ERAP2	426	PRKCSH	498	HRSP12
67	Ankrd26	139	C1qB	211	CFI1	283	Cytokeratin 16	355	Erp29	427	GLUD1	499	HSC70
68	Annexin A1	140	C1qR1	212	CFVII	284	Cytokeratin 17	356	Erp57	428	CGH	500	HSP47
69	Annexin A2	141	C1RL	213	CHC17	285	Cytokeratin 20	357	Erp72	429	GSTO1		
70	Annexin A6	142	C1s	214	Chitobiasase	286	Cytokeratin 3	358	ESD	430	GSS		
71	Annexin V	143	ELP6	215	Chitotriosidase	287	Cytokeratin 4	359	ESR1	431	GPD1		
72	ANP	144	C4.4A	216	CHORDC1	288	Cytokeratin 5	360	ETL	432	Glycoprotein V		

# D. RayBio® Human Antibody Array L-4 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	HEXA	73	LIMS1	145	Nectin-1	217	Peroxiredoxin 3	289	PTK 7	361	Serpin A7	433	Thymosin b10
2	HTRA1	74	LMAN2	146	Nectin-3	218	Peroxiredoxin 5	290	PTMA	362	Serpin B3	434	Titin
3	Agrin	75	ACP1	147	NEDD8	219	PF4V1	291	PTP gamma	363	Serpin B6	435	TLS
4	IBP160	76	LOK	148	Neogenin	220	PGAM1	292	PTP kappa	364	Serpin B8	436	TMEM223
5	IDH1	77	LOX	149	Nesprin2	221	PGAM2	293	PTP mu	365	Serpin F2	437	TOB2
6	IDH3A	78	LOXL1	150	Neurabin 1	222	PGD	294	PTPRS	366	Serpin A10	438	TOP2B
7	IFRD1	79	LRP 4	151	Neural Cadherin	223	PHGDH	295	PTRZ	367	SERPINB1	439	TPM4
8	IGF2BP2	80	LTA4H	152	PAM	224	PGK-1	296	PYGL	368	SerpinB4	440	TPP1
9	ITGB5	81	LTBP4	153	Neurogranin	225	PGLS-C-t	297	PZP	369	SerpinE2	441	TALDO1
10	IGSF4B	82	Lubricin	154	Neuropeptide B	226	PGM1	298	QDPR	370	SerRS	442	TALDO
11	Ihh	83	LUZP1	155	Neuropilin-1	227	PGRPL	299	QPRT	371	SET	443	Transthyretin
12	ILK	84	LYPA1	156	Neurotrimin	228	PHAP1	300	Quiescin Q6	372	SEZ6L2	444	TRAP1
13	Inhibin beta	85	Lysozyme	157	NF-M	229	PSAT1	301	Rab7a	373	SF20	445	TRAP220
14	ITGB1	86	MAGI2	158	Nidogen-2	230	PIK3C2B	302	Ran	374	SH3BGRL	446	TRF 2
15	ITGB6	87	MAGP-2	159	NIT2	231	plgR	303	RanGAP1	375	SH3BGRL3	447	TPIS
16	ITGA6	88	MAN1	160	NME3	232	PIK3IP1	304	RAP1AB	376	SHANK1	448	Tropomyosin 3
17	IQGAP1	89	MANF	161	nNOS	233	PIN	305	Rbm15	377	SHC1	449	Twist-1
18	IQGAP2	90	Mannosidase II	162	Noelin	234	PISD	306	RCL	378	SHIP	450	TRPS1
19	IRE1	91	MAP1A	163	Non-muscle Actin	235	PKLR	307	Reg1A	379	SHMT1	451	Trypsinogen-2
20	IRS2	92	MAPRE1	164	Myosin IIA	236	PLA2G1B	308	Reg3A	380	SHP-1	452	Trypsin Pan
21	ISOC2	93	MARCKS	165	Notch-2	237	Plakophilin 1	309	RHOC	381	Siglec-1	453	WRS
22	ITGB4BP	94	MASP3	166	Notch-2 1CD	238	Plastin L	310	RhoGDI	382	SIGLEC14	454	TSR2
23	ITIH1	95	MBD2	167	NPAS3	239	PLC-gamma 1	311	RNASE1	383	SIM2	455	TUBA6
24	ITIH2	96	MBP	168	NPM1	240	Pleckstrin	312	RNH1	384	SIRP beta 1	456	TWF2
25	ITIH3	97	MCAM	169	NQO2	241	Plectin	313	RNASET2	385	Six3	457	TXNDC4
26	ITIH4 a	98	Mcl-1	170	NT5C3	242	Plexin B1	314	RKIP	386	SLC38A10	458	TXNDC5
27	JAM-A	99	MCM	171	NUCB1	243	Plexin B2	315	POLR2A	387	SLITRK1	459	TXNRD2
28	JARID2	100	MCM5	172	NUP98	244	PLD1	316	RNASE4	388	SLURP1	460	UBE2D3
29	KPNB1	101	MCMP2	173	OBCAM	245	PLD2	317	RNASE6	389	SMA	461	Ube2L3
30	Keratin 36	102	MDH1	174	OIT3	246	PLS3	318	RPL10	390	SMC4	462	UBE2N
31	Keratin 38	103	MDH2	175	Olfactomedin-2	247	Pixdc2	319	RPL10A	391	SMPD4	463	Ubiquitin
32	KHSRP	104	ME1	176	OTC	248	PNP	320	RPL11	392	SOD1	464	UCH-L1
33	KIAA0319L	105	MEP1A	177	Orosomucoid 2	249	POR	321	RPL12	393	SOD2	465	UFM 1
34	KIAA1468	106	Metallothionein	178	ORP150	250	PPCS	322	RPL14	394	SOD-3	466	UGGT
35	KIAA1967	107	Metavinculin	179	OSBP1	251	PPOX	323	RPL17	395	SOD4	467	UNC13D
36	KIF5B	108	MFAP4	180	OSCAR	252	PPP2R1B	324	RPL22	396	Somatostatin	468	UNC45A
37	Kilon	109	MF12	181	OSM R beta	253	PPP2R4	325	RPL5	397	SORD	469	UNC5H4
38	KLK-B1	110	mGLUR5	182	Osteoadherin	254	PRCP	326	RPL7A	398	SorLA	470	UPB1
39	KMD4B	111	MGP	183	OXT	255	PRDM13	327	RPLP0	399	SOX4	471	UQCRB
40	KMT2B	112	Mimecan	184	p16 ARC	256	PRDX 1	328	RPS10	400	SP-D	472	UQCRH
41	KRT31	113	MINPP1	185	P205b3	257	PRELP	329	RPS11	401	Spectrin beta-5	473	URB
42	KRT72	114	MLCK	186	p23	258	PREP	330	RPS12	402	SPEN	474	URB2
43	Krt73	115	MMR	187	p39	259	PRG2	331	RPS19	403	SPINK7	475	UROC1
44	KRT82	116	MMRN1	188	P4HB	260	PRNP	332	RPS2	404	SPTBN1	476	UROD
45	KRT85	117	MN1	189	p73	261	Profilin 1	333	RPS20	405	Src	477	URP2
46	KRTDAP	118	Moesin	190	PA2G4	262	Properdin	334	RPS23	406	SREC-II	478	USP14
47	KRTHA3B	119	MP1	191	PABP	263	Prosaoposin	335	RPS25	407	STAT3	479	USP5
48	KSR1	120	MPCA	192	PACS1	264	PTGDS	336	RPS28	408	Stathmin 1	480	Uteroglobin
49	LAD	121	MPO	193	PARVB	265	PSMB6	337	RPS3	409	SCP2	481	Utrophin
50	LAF4	122	MRP 1	194	PCBP1	266	PSMA3	338	RPS5	410	STI1	482	VAP-1
51	LAIR1	123	MSH6	195	PCBP2	267	PSMA5	339	RREB1	411	STOM	483	VAP-A
52	LAM b1	124	mTOR	196	PCCA	268	PSMB7	340	RSU1	412	SUCLG1	484	VCP
53	LAMA	125	MUCDHL	197	PCDH7	269	PSMD5	341	S100A1	413	SUMO3	485	VDAC1
54	LMNA	126	Multimerin 2	198	PCDX8	270	PSMB1	342	S100A11	414	Symplekin	486	Versican
55	LMNB1	127	MyBPC3	199	PCK2	271	PSMA6	343	S100A7	415	SynCAM	487	Vimentin B
56	LMNB2	128	MYH2	200	PCMT1	272	PSB2	344	S100A9	416	Syntaxin 7	488	VNN1
57	LAMA2	129	MYH6	201	PCNA	273	PSB4	345	S100P	417	TAB182	489	VSG4
58	LAMB2	130	MYH7	202	PCPE-1	274	Protein C	346	TIM-4	418	TAGLN2	490	WDR1
59	LAMC1	131	MYHC	203	PCSK9	275	Protein Z	347	SAA4a	419	Talin1	491	WISP2
60	LAMP	132	MYL12B	204	PCYOX1	276	Prourguanylin	348	aAmylase	420	Talin1&2	492	WNK2
61	LAMP1	133	MYL3	205	PDE1B	277	PRSS23	349	SAMSNI	421	TAX1BP3	493	YB1
62	LAMP2	134	MYO5A	206	PDIA6	278	PRSS3	350	SBP-1	422	TBCA	494	YY1
63	LAP3	135	Myoferlin	207	PDLIM1	279	PRTN3	351	SBSN	423	TCEB2	495	ZBTB4
64	LASP1	136	Myosin 18B	208	PDLIM5	280	PSMA1	352	SDF4	424	Tcf20	496	ZC3H4-N-t
65	LTBP2	137	Myotrophin	209	PDZD2	281	PSMA2	353	SDNSF	425	TCN1	497	ZC3H8
66	LCAT	138	NABC1	210	PEBP4	282	PSMA4	354	SDPR	426	TCP1 eta	498	ZDHHC18
67	LCMT2	139	NAGLU	211	PEPD	283	PSMA7	355	SCG5	427	Tenascin C	499	ZNF671
68	LDHA	140	NAP1L1	212	PER1	284	PSMB5	356	Semaphorin 6B	428	Tenascin X	500	Zyxin
69	LDHB	141	NAPRT1	213	perilipin 3	285	PSMC3	357	Semaphorin 7A	429	TFF2		
70	LEDGF	142	NASP	214	Perilipin-1	286	PSMD1	358	SEM6G1	430	TGM3		
71	SPINK5	143	NCAM2	215	Periostin	287	PSMD9	359	SEM6G2	431	Thioredoxin-1		
72	LILRA3	144	Nebulin	216	Peroxiredoxin 2	288	PTEN	360	Serpin A11	432	THOP1		

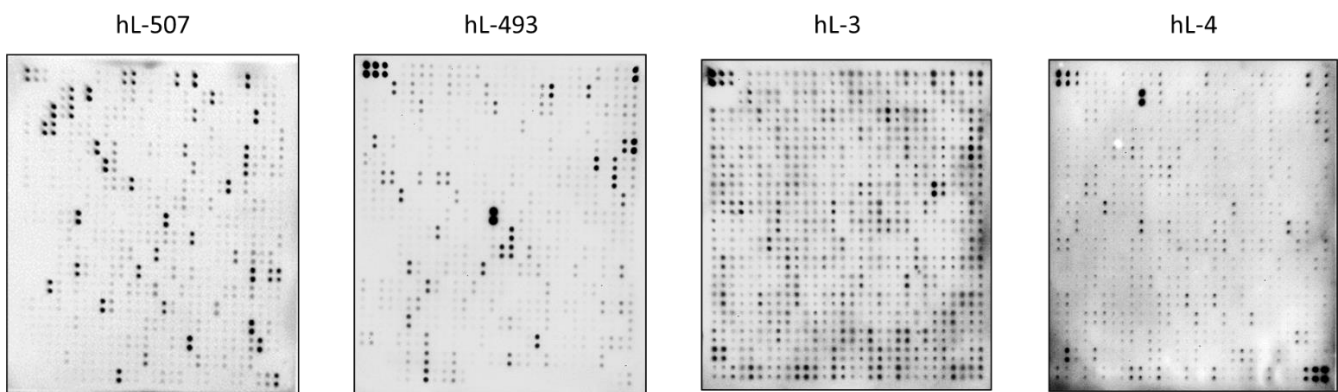
## VII. Interpretation of Results:

### A. Explanation of Controls Spots

To obtain optimal results using a chemiluminescence imaging system (UVP BioImaging Systems), it is suggested to try several different exposure times until the best one is determined. Then, by comparing the signal intensities, relative expression levels of the target proteins can be made. The intensities of signals can be quantified by densitometry. There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized anti-HRP antibodies, which will produce positive control signals after incubation with HRP-conjugated Streptavidin. With all other variables being equal, the Positive Control intensities will be the same for each sub-array, which allows for inter-array normalization. Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies. Some arrays may have beta-actin and GAPDH as internal controls, much as “housekeeping” genes or proteins are used to normalize results in PCR or Western blots, respectively.

### B. Typical Results

The following figure shows the typical result of arrays probed with human serum sample.



*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 (ie, 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  $\geq 1.5$ -fold increase or  $\leq 0.65$ -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  $\approx 95\%$ ).

### **F. Pathway Analysis of the Array Proteins**

Human antibody array L-2000 detects 2000 unique human proteins, including most analyzed cytokines, chemokines, adipokines, extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins. All the array proteins are provided with their Uniprot number and GeneID, which are essential for further data mining. Raybiotech offers affordable biostatistics and bioinformatics service, including data clean-up, differential expression analysis, cluster analysis, biomarker selection, pathway analysis and experimental design. See more details on the website: <https://www.raybiotech.com/biostatistics-and-bioinformatics-services>

## VIII. Troubleshooting Guide

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>	
<b>Weak Signal</b>	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 -20C and avoid free 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.			
<b>Uneven signal</b>	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.	
<b>High background</b>	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.	

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