

RayBio[®] Label-Based (L-Series)

Human Antibody Array L-2000 Glass Slide 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 or other body fluids.

L-Series Human Antibody Array L-2000
Cat# AAH-BLG-2000-4 (4 Sample Kit)
Cat# AAH-BLG-2000-8 (8 Sample Kit)

Please read manual carefully
before starting experiment



Your Provider of Excellent Protein Array Systems and Services

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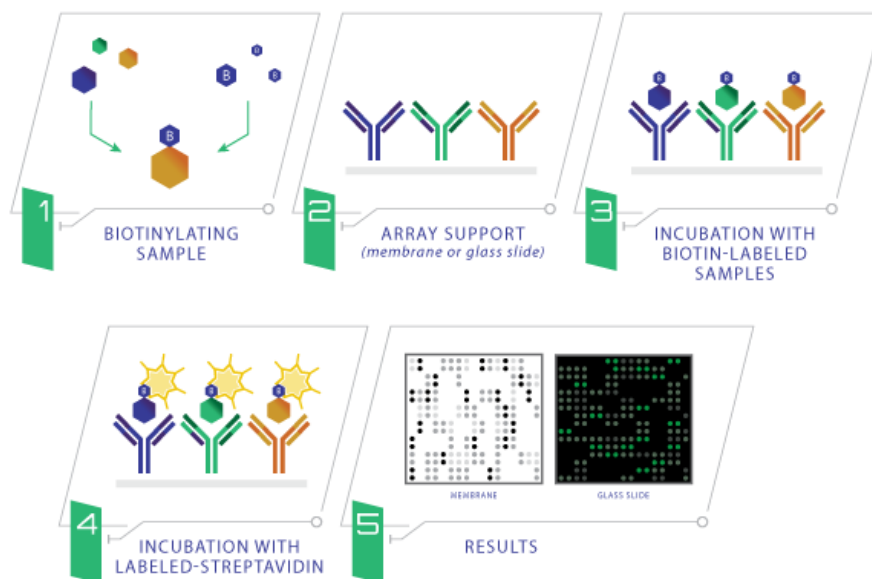
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I. Introduction

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

The first step in using the RayBio® L-Series Antibody Array is to biotinylate the primary amine groups of the proteins in your sample (sera or plasma, cell culture supernatants, cell lysates or tissue lysates). The glass slide arrays are then blocked, just like a western blot, and the biotin-labeled sample is added onto the glass slide, which is pre-printed with capture antibodies. The slide is incubated to allow binding of target proteins. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then applied to the array. Finally, the glass slide is dried, and laser fluorescence scanning is used to visualize the signals.



II. Materials Provided

A. Storage Recommendations

Upon receipt, the kit should be stored at -20°C until needed. It is recommended to use the kit within 6 months of the date of shipment. After initial use, remaining reagents should be stored at 4°C and may be stored for up to 3 months. Labeling Reagent (Item B) should be prepared fresh each time before use. Unused glass slides should be kept at -20 °C and repeated freeze-thaw cycles should be avoided (slides may be stored for 6 months).

ITEM	DESCRIPTION	4 SAMPLE Kit	8 SAMPLE Kit
A	Spin Columns (0.5ml)	16 columns	32 columns
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	2 vials (50 µl)
E	RayBio® L-Series Glass Slide*	1 slide each of Human L-507, L-493, L-3, and L-4	2 slides each of Human L-507, L-493, L-3, and L-4
F	Blocking Buffer	1 bottle (30 ml)	2 bottles (30 ml)
G	20X Wash Buffer I	2 bottles (30 ml)	3 bottles (30 ml)
H	20X Wash Buffer II	2 bottles (30 ml)	3 bottles (30 ml)
I	Cy3 equivalent-Conjugated Streptavidin	2 vials	3 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (30 ml)	2 bottles (30 ml)
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	1 bottle (10 ml)
M	30 ml Centrifuge Tube	1 tube	2 tubes

*Each slide contains 4 identical subarrays

**Only needed if testing cell or tissue lysates

B. Additional Materials Required

- 1 ml tube, small plastic or glass containers
- Orbital shaker or oscillating rocker
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection
- Aluminum foil

III. Overview and General Considerations

A. Preparation and Storage of Samples

1) Preparation of Cell Culture Supernatants

1. Seed cells at a density of 1×10^6 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.**,† The membrane-based array is recommended if high serum medium such as 10% FCS/FBS is used, as high background can occur on glass slide arrays with high serum containing media samples.
4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as ≤ 1 ml aliquots at -80°C until needed.
5. If you want to use cell mass for inter-sample normalization, measure the total wet weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can normalize between arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

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

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

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

2) Extracting Protein from Cells

1. Centrifuging Cells

a. Adherent Cells:

- i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.
- ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.

b. Cells in Suspension: Pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 minutes.

2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH₂O). Solubilize the cells at 2×10^7 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 minutes at 2-8 °C.

Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer lysates to a clean tube. Determining cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.

3) Extracting Protein from Crude Tissue

1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH₂O).

2. Homogenize the tissue according to homogenizer manufacturer instructions.
3. Transfer extracts to microcentrifuge tubes and centrifuge for 20 minutes at 13,000 rpm (4°C).

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

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

4) Determine the total protein concentration

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

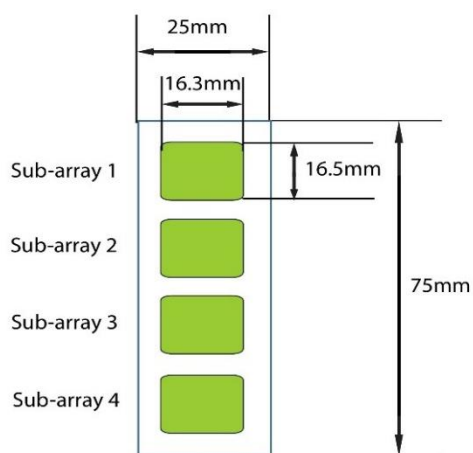
B. Handling the Glass Slides

- The microarray slides are delicate. Please do not touch the array surface with pipette tips, forceps or your fingers. Hold the slides by the edges only.
- Handle the slides with powder-free gloves and in a clean environment.
- Do not remove the glass slide from the chamber assembly until step 20, and take great care not to break the glass slide when doing so.
- Remove reagents/sample by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides as seen in image below.



C. Layout of Array Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

D. Incubations and Washes

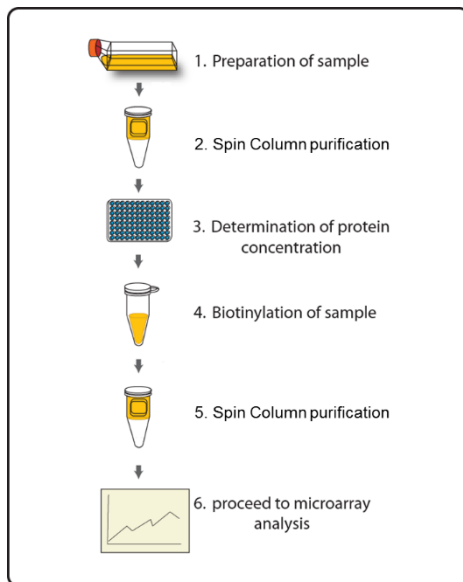
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.

- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 4°C.
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.
- Unlike most Cy3 fluors, the streptavidin-conjugated fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from direct, strong light and temperatures above RT.

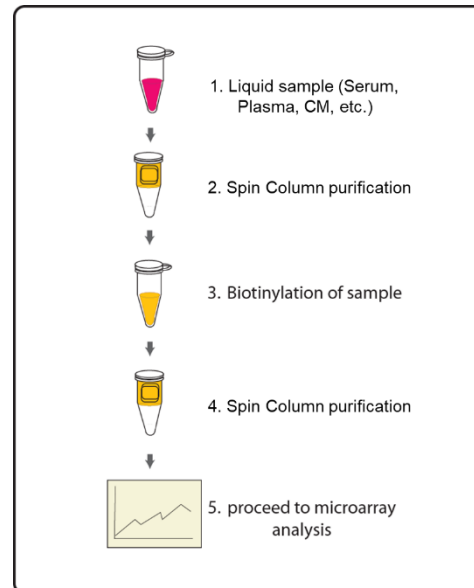
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



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



A. Sample purification

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

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).
2. Place the Spin column into a collection tube, centrifuge at 1,500 x g for 1 minute to remove the storage buffer. Discard the flow-through.
3. Wash the Spin Column three times with 300 μ l Labeling Buffer each, centrifuge at 1,500 x g for 1 minute to remove the flow-through. Discard the flow-through and blot the bottom of the column to

remove excess liquid. Transfer the Spin Column to a new collection tube.

4. Apply sample on top of the resin within the next few minutes. Centrifuge at 1,500 x g for 2 minutes. Collect the flow-through that contains the sample. The recommended sample dilutions are as follows:

- *Culture Media: 120 μ l neat supernatant*
- *Serum/Plasma: 2 μ l serum/plasma in 100 μ l labeling buffer*
- *Cell/tissue lysate: 20 μ g lysate in 100 μ l labeling buffer*

Note: Each labelled sample volume is enough for at least 3 arrays following the protocol below.

Note: The maximal sample volume is 130 μ l for each Spin Column. Do not load over 130 μ l of sample into a Spin Column.

B. Biotin-Labeling the Sample

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

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 μ l 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 8 μ l of Labeling Reagent into the sample tube (for 120 μ l supernatant).

- b. For labeling serum or plasma: Add 8 μ l of Labeling Reagent Solution into the sample tube (for 2 μ l serum/plasma *in 100 μ l labeling buffer*).
- c. For labeling cell or tissue lysates: Add 4 μ l of Labeling Reagent Solution into the sample tube (for 20 μ g lysate *in 100 μ l labeling buffer*).
- d. For all other body fluid: Add 2 μ l of Labeling Reagent Solution per 100 μ g sample to be labelled.

Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If the amount of sample being labelled differs from the example in Step 6, adjust this volume proportionally.

7. Add 3 μ l Stop Solution (Item D) to each sample tube. Using a new spin column, repeat Steps 1-4 of section A. Sample Purification to remove the excess non-reacted biotin reagent from each sample.

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

C. Drying the Glass Slide

8. Remove the package containing the Assembled Glass Slide (Item E) from the freezer. Place unopened package on the bench top for ~15 minutes, and allow the Assembled Glass Slide to equilibrate to RT.
9. Open package, and take the Assembled Glass Slide out of the sleeve. Do not disassemble the Glass Slide from the chamber assembly. Place glass slide assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubations

Note: Glass slide should be completely dry before adding Blocking Buffer to wells.

10. Block sub-arrays by adding 400 μ l of Blocking Buffer (Item F) into each well of Assembled Glass Slide and incubating at RT for 30 minutes. Ensure there are no bubbles on the array surfaces.
11. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 10-fold for cell culture supernatants, 20-fold for serum/plasma and 100-fold for cell/tissue lysate. *Dilution for other body fluid needs to be determined by the end user. Generally, most samples can be 10-20x dilution, while tears and saliva samples may need 100x dilution.*

Note: Optimal sample dilution factor will depend on the abundance of target proteins. If the background or antigen-specific antibody signals are too strong, the sample can be diluted further in subsequent experiments. If the signal is too weak, more concentrated samples can be used.

12. Completely remove the Blocking Buffer from each well. Add 400 μ l of diluted sample into appropriate wells. Remove any bubbles on array surfaces. Incubate arrays with gentle rocking or shaking for 2 hours at RT or overnight at 4°C.

Note: Avoid the flow of sample into neighboring wells.

13. Based on number of samples and remaining protocol, calculate the amount of 1X Wash Buffer I and 1X Wash Buffer II needed to complete the experiment. Separately dilute the required amounts of 20X Wash Buffer I Concentrate (Item G) 20-fold and 20X Wash Buffer II Concentrate (Item H) with ddH₂O.
14. Decant the samples from each well and wash 3 times with 800 μ l of 1X Wash Buffer I at RT with gentle rocking or shaking for 5 minutes per wash.

15. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer I to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 10 minutes per wash.
16. Decant the Wash Buffer I from each well, place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer II to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 5 minutes per wash.
17. Prepare 1X Cy3-Conjugated Streptavidin:
 - a) Briefly spin down tube containing the Cy3-Conjugated Streptavidin (Item I) immediately before use.
 - b) Add 1000 μ l of Blocking Buffer into the Cy3-Conjugated Streptavidin tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).
 - c) To prepare 1X Cy3-Conjugated Streptavidin, add 200 μ l of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800 μ l of Blocking Buffer. Mix gently.
18. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400 μ l of 1X Cy3-Conjugated Streptavidin to each sub-array. Cover the incubation chamber with the plastic adhesive strips.

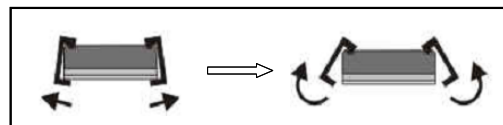
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 1 hour with gentle rocking or shaking.

Note: Incubation may be done overnight at 4°C.

20. Decant the solution and disassemble the glass slide from the incubation frame and chamber. Disassemble the device by pushing clips outward from the side, as shown below. Carefully remove the glass slide from the gasket.

Note: Be careful not to touch the printed surface of the glass slide, which is on the same side as the barcode.



21. Gently place the glass slide into 30 ml Centrifuge Tube (Item M). Add enough 1X Wash Buffer I to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 10 min. Remove the wash buffer. Repeat 2 times for a total of 3 washes.
22. Add enough 1X Wash Buffer II to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 5 minutes. Remove the wash buffer. Repeat one time for a total of two washes for 5 minutes per wash.
23. Finally, wash the glass slide with 30 ml of ddH₂O for 5 minutes. Remove glass slide and decant water from Centrifuge Tube.
24. Remove buffer droplets from the slide completely by one of the following ways:
 - Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
 - Or dry the glass slide by a compressed N₂ stream.
 - Or gently apply suction with a pipette to remove buffer droplets. Do not touch the array surface, only the sides.

Note: Make sure the finished glass slide is completely dry before scanning or storage.

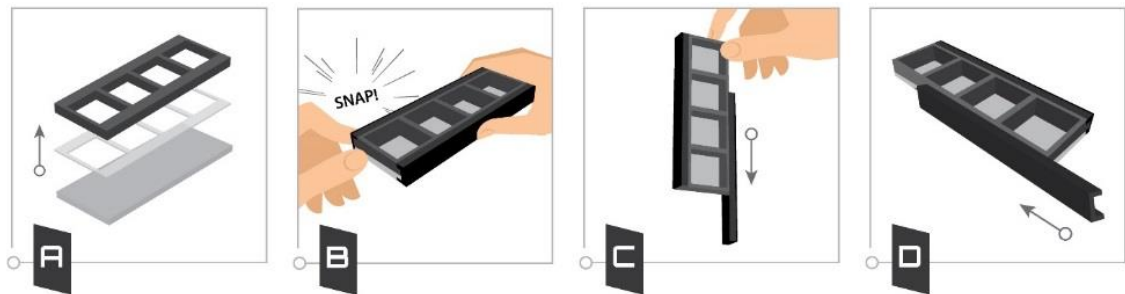
E. Fluorescence Detection

25. You may proceed immediately to scanning or you may store the slide at -20 °C in the Centrifuge Tube provided or at RT to scan at a later time.

Note: Please protect the finished glass slides from temperatures above RT and store them in the dark. Do not expose glass slide to strong light, such as sunlight or a UV lamp.

Note: If you need to repeat any of the incubation steps after finishing the experiment, you must first re-assemble the glass slide into the incubation chamber by following the steps as described below. To avoid breaking the printed glass slide, you may first want to practice assembling the device with a blank glass slide.

1. Apply slide to incubation chamber barcode facing upward (image A).
2. Gently snap one edge of a snap-on side (image B).
3. Gently press other of side against lab bench and push in lengthwise direction (image C).
4. Repeat with the other side (image D)



V. Antibody Array Map

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
1	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11
2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
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35	503	503	504	504	505	505	506	506	507	507	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS1	POS1	POS2	POS2	POS3	POS3	

B. RayBio® Human Antibody Array L-493 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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2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
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4	42	42	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56
5	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71
6	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86
7	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101
8	102	102	103	103	104	104	105	105	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116
9	117	117	118	118	119	119	120	120	121	121	122	122	123	123	124	124	125	125	126	126	127	127	128	128	129	129	130	130	131	131
10	132	132	133	133	134	134	135	135	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146
11	147	147	148	148	149	149	150	150	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160	161	161
12	162	162	163	163	164	164	165	165	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176
13	177	177	178	178	179	179	180	180	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191
14	192	192	193	193	194	194	195	195	196	196	197	197	198	198	199															

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	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	
2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26	
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17	237	237	238	238	239	239	240	240	241	241	242	242	243	243	244	244	245	245	246	246	247	247	248	248	249	249	250	250	251	251	
18	252	252	253	253	254	254	255	255	256	256	257	257	258	258	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266	
19	267	267	268	268	269	269	270	270	271	271	272	272	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280	281	281	
20	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	282	282	283	283	284	284	285	285	286	286	287	287	288	288	289	289	290	290	291	291	292	292	
21	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300	301	301	302	302	303	303	304	304	305	305	306	306	307	307	
22	308	308	309	309	310	310	311	311	312	312	313	313	314	314	315	315	316	316	317	317	318	318	319	319	320	320	321	321	322	322	
23	323	323	324	324	325	325	326	326	327	327	328	328	329	329	330	330	331	331	332	332	333	333	334	334	335	335	336	336	337	337	
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32	458	458	459	459	460	460	461	461	462	462	463	463	464	464	465	465	466	466	467	467	468	468	469	469	470	470	471	471	472	472	
33	473	473	474	474	475	475	476	476	477	477	478	478	479	479	480	480	481	481	482	482	483	483	484	484	485	485	486	486	487	487	
34	488	488	489	489	490	490	491	491	492	492	493	493	494	494	495	495	496	496	497	497	498	498	499	499	500	500	Neg	Neg	Neg	Neg	
35	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

VI. Antibody Array Target List

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

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	6CKine	74	F3	147	FGF-19	220	IGFBP-4	293	IL-22 BP	366	MMP-20	439	Shh-N
2	Activin A	75	CRIM 1	148	FGF-20	221	IGFBP-6	294	IL-22 R	367	MMP-24	440	SPARC
3	Activin B	76	Cripto-1	149	FGF-21	222	IGFBP-rp1	295	IL-23	368	MMP-25	441	Spinesin
4	Activin C	77	CRTH-2	150	FGF-23	223	IGF-I	296	IL-23 R	369	MSPa	442	TACI
5	Activin RIA	78	Cryptic	151	FLRG	224	IGF-I R	297	IL-24	370	Musk	443	Tarc
6	Activin RIB	79	Csk	152	Flt-3 Ligand	225	IGF-II	298	IL-26	371	NAP-2	444	TECR
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	Neurtin	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	Nidogen-1	451	ATP2B1
14	Angiopoietin-2	87	CXCR2	160	Frizzled-6	233	IL-1 F9	306	Insulin	379	NOV	452	TGF-beta RI
15	Angiopoietin-4	88	CXCR3	161	Frizzled-7	234	IL-1 F10	307	Insulin R	380	NrCam	453	TGF-beta RII
16	ANGPTL1	89	CXCR4	162	Galectin-3	235	IL-1 R3	308	Insulysin	381	GGF2	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	APRIL	94	DANCE	167	G-CSF R	240	IL-1 ra	313	Kremen-2	386	Orexin A	459	Thrombospondin-2
22	Amphiregulin	95	DCR3	168	GDF1	241	IL-1 RI	314	LTBP1	387	Orexin B	460	Thrombospondin-4
23	Artemin	96	Decorin	169	GDF3	242	IL-1 RII	315	LBP	388	OSM	461	Thymopoietin
24	Axl	97	Dkk-1	170	GDF5	243	IL-2	316	Lck	389	Osteoactivin	462	Tie-1
25	B7-1	98	Dkk-3	171	GDF8	244	IL-2 R alpha	317	LECT2	390	Osteocrin	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	392	OX40 Ligand	465	TIMP-2
28	BD-1	101	DR6	174	GDF-15	247	IL-3	320	Leptin R	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	Lipocalin-2	399	PDGF-BB	472	TLR4
35	BMP-2	108	EG-VEGF	181	GITR Ligand	254	IL-6 R	327	LRP-1	400	PDGF-C	473	TMEFF1
36	BMP-3	109	EMAP-II	182	CBR1	255	IL-7	328	LRP-6	401	PDGF-D	474	TMEFF2
37	BMP-3b	110	ENA-78	183	Glut1	256	IL-7 R alpha	329	L-Selectin	402	PECAM-1	475	TNF-alpha
38	BMP-4	111	Endocan	184	Glut2	257	IL-8	330	Lymphotactin	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	Endothelin	187	Glypican 3	260	IL-10 R alpha	333	MAC-1	406	PIGF	479	TRADD
42	BMP-8	115	EN-RAGE	188	Glypican 5	261	IL-10 R beta	334	MCP-1	407	PLUNC	480	TRAIL
43	BMP-15	116	Eotaxin	189	GM-CSF	262	IL-11	335	MCP-2	408	Pref-1	481	TRAIL R1
44	BMPR-IA	117	Eotaxin-2	190	GM-CSF R alpha	263	IL-12 p40	336	MCP-3	409	Progranulin	482	TRAIL R2
45	BMPR-IB	118	Eotaxin-3	191	Granzyme A	264	IL-12 p70	337	MCP-4	410	Prolactin	483	TRAIL R3
46	BMPR-II	119	Epregrulin	192	GREMLIN	265	IL-12 R beta 1	338	M-CSF	411	P-selectin	484	TRAIL R4
47	BTC	120	ErbB2	193	GRO	266	IL-12 R beta 2	339	M-CSF R	412	RAGE	485	TRANCE
48	Cardiotrophin-1	121	ErbB3	194	GRO-a	267	IL-13	340	MDC	413	RANK	486	TREM-1
49	CCL14	122	ErbB4	195	GH	268	IL-13 R alpha 1	341	MFG-E8	414	RANTES	487	TROY
50	CCL28	123	Erythropoietin	196	GHR	269	IL-13 R alpha 2	342	MFRP	415	RELN beta	488	TSG-6
51	CCR1	124	E-Selectin	197	HB-EGF	270	IL-15	343	MICA	416	RELT	489	TSLP R
52	CCR2	125	FADD	198	HCC-4	271	IL-15 R alpha	344	MIF	417	ROBO4	490	TWEAK
53	CCR3	126	FAM3B	199	HCR	272	IL-16	345	MIG	418	S100 A8/A9	491	TWEAK R
54	CCR4	127	Fas	200	Hepassocin	273	IL-17	346	MIP-1a	419	S100A10	492	Ubiquitin+1
55	CCR5	128	Fas Ligand	201	GLO-1	274	IL-17B	347	MIP-1b	420	SAA	493	uPA
56	CCR6	129	FGF Basic	202	HGF	275	IL-17B R	348	MIP-1d	421	SCF	494	uPAR
57	CCR7	130	FGF-BP	203	HGFR	276	IL-17C	349	MIP 2	422	SCF R	495	Vasorin
58	CCR8	131	FGF R3	204	HRG-alpha	277	IL-17D	350	MIP-3 alpha	423	SDF-1	496	VCAM-1
59	CCR9	132	FGF R4	205	HRG-beta 1	278	IL-17E	351	MIP-3 beta	424	sFRP-1	497	VE-Cadherin
60	CD14	133	FGF R5	206	HVEM	279	IL-17F	352	MMP-1	425	sFRP-3	498	VEGF
61	CD27	134	FGF-4	207	I-309	280	IL-17R	353	MMP-2	426	sFRP-4	499	VEGF R2
62	CD30	135	FGF-5	208	ICAM-1	281	IL-17RC	354	MMP-3	427	sgp130	500	VEGF R3
63	CD30 Ligand	136	FGF-6	209	ICAM-2	282	IL-17RD	355	MMP-7	428	SIGIRR	501	VEGF-B
64	CD40	137	FGF-7	210	ICAM-3	283	IL-18 BPa	356	MMP-8	429	Siglec-5	502	VEGF-C
65	CD40 Ligand	138	FGF-8	211	ICAM-5	284	IL-18 R alpha	357	MMP-9	430	Siglec-9	503	VEGF-D
66	CD 163	139	FGF-9	212	IFN-alpha/beta R1	285	IL-18 R beta	358	MMP-10	431	SLPI	504	VEGI
67	Cerberus 1	140	FGF-10	213	IFN-alpha/beta R2	286	IL-19	359	MMP-11	432	Smad 1	505	WIF-1
68	Chem R23	141	FGF-11	214	IFN-beta	287	IL-20	360	MMP-12	433	Smad 4	506	WISP-1
69	Chordin-Like 1	142	FGF-12	215	IFN-gamma	288	IL-20 R alpha	361	MMP-13	434	Smad 5	507	XEDAR
70	Chordin-Like 2	143	FGF-13 1B	216	IFN-gamma R1	289	IL-20 R beta	362	MMP-14	435	Smad 7		
71	CLC	144	FGF-16	217	IGFBP-1	290	IL-21	363	MMP-15	436	Smad 8		
72	CNTF	145	FGF-17	218	IGFBP-2	291	IL-21 R	364	MMP-16	437	Prdx6		
73	CNTF R alpha	146	FGF-18	219	IGFBP-3	292	IL-22	365	MMP-19	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	BLAME	145	C-peptide	217	FoxO1	289	KIF3B	361	PTH	433	Serpin G1
2	2B4	74	BMP-9	146	Creatinine	218	FoxP3	290	KLF4	362	Troponin C	434	SERTAD2
3	4-1BB	75	BMX	147	CRP	219	FRK	291	LAG-3	363	PDX-1	435	SHBG
4	ABL1	76	BNIP2	148	CRTAM	220	ARB1	292	pro-Glucagon	364	PEDF	436	SMAC
5	ACE	77	Btk	149	CSH1	221	Furin	293	Layilin	365	PEPSINOGEN I	437	SNCG
6	ACE-2	78	ApoC1	150	gamma-Thrombin	222	Fyn	294	LDL R	366	PEPSINOGEN II	438	SSTR5
7	ACK1	79	CA9	151	CutA	223	GADD45A	295	Legumain	367	Vasopressin	439	SCGF
8	ACPP	80	CA15-3	152	Troponin T	224	Galectin-1	296	LH	368	PGRP-S	440	SOST
9	ACTH	81	CA19-9	153	Cyclin D1	225	Galectin-3BP	297	LIMP1	369	PI 16	441	SOX17
10	ADAM-9	82	CA125	154	Cystatin A	226	Galectin-7	298	LIN41	370	PIK3R1	442	SOX2
11	Neurokinin A	83	Cadherin-13	155	Cystatin B	227	Gas1	299	Livin	371	PIM2	443	SPARCL1
12	ADAMTS1	84	CLEC14A	156	Cystatin C	228	Gastrin	300	LOX-1	372	PKM2	444	SPINK1
13	ADAMTS12	85	Calbindin D	157	Cytochrome C (d)	229	GATA-3	301	LPS	373	Plasminogen	445	SRMS
14	ADAMTS4	86	Calcitonin	158	Cytokeratin 8	230	GATA-4	302	LRG1	374	Podocalyxin	446	SSEA-1
15	ADAMTS5	87	Calreticulin	159	Cytokeratin 18	231	Gelsolin	303	LTf	375	POMC	447	SSEA-4
16	ADAMTS10	88	Calsyntenin-1	160	Cytokeratin 19	232	Ghrelin	304	LTK	376	PON1	448	SSTR2
17	ADAMTS13	89	CPN2	161	DBI	233	GLP-1	305	Lumican	377	PON2	449	Survivin
18	ADAMTS15	90	CART	162	DCBLD2	234	GPI	306	Lyn	378	PPARg2	450	SYK
19	ADAMTS17	91	Caspase-3	163	D-Dimer	235	GPBB	307	LYRIC	379	PPP2R5C	451	Syndecan-1
20	ADAMTS18	92	Caspase-8	164	DEFA1/3	236	GMNN	308	LYVE-1	380	NR3C3	452	Syndecan-3
21	ADAMTS19	93	Cathepsin B	165	CPA1	237	GPR-39	309	LZTS1	381	INSL3	453	TACE
22	Adipsin	94	Cathepsin D	166	Desmin	238	GPX1	310	Mammaglobin A	382	Pro-BDNF	454	TAFA4
23	Afamin	95	Cathepsin L	167	DLL1	239	GPX3	311	Marapsin	383	Procalcitonin	455	Tyk2
24	AFP	96	Cathepsin S	168	DLL4	240	Pancreastatin	312	MATK	384	Pro-Cathepsin B	456	Tec
25	ALBUMIN	97	CBP	169	DMP-1	241	GRP	313	MBL	385	Thrombin	457	TFF3
26	IL-28B	98	CCK	170	DPPIV	242	GRP75	314	C1qTNF1	386	Prohibitin	458	Thrombomodulin
27	Aldolase A	99	CD23	171	BNP	243	GRP78	315	Mer	387	ProSAAS	459	TK1
28	Aldolase B	100	CD24	172	E-Cadherin	244	GSR	316	Mesothelin	388	Prostasin	460	Thyroglobulin
29	Aldolase C	101	CD36	173	Endorphin Beta	245	GST	317	MICB	389	PSP	461	TIM-1
30	ALK	102	CD38	174	EDNRA	246	HADHA	318	Midkine	390	Pro-MMP-7	462	TNK1
31	Alpha Lactalbumin	103	CD44	175	Enolase 2	247	HAI-1	319	MINA	391	Pro-MMP-9	463	TOPORS
32	Alpha 1 AG	104	CD45	176	ENPP2	248	HAI-2	320	FABP3	392	Protein p65	464	TPA
33	A1BG	105	CD46	177	EpCAM	249	hCG alpha	321	MSHa	393	PSA-Free	465	TRA-1-60
34	A1M	106	CD47	178	EphA1	250	hCgb	322	MTUS1	394	PSA-total	466	TRA-1-81
35	A2M	107	CD55	179	EphA2	251	Hck	323	Myoglobin	395	PTHLP	467	Transferrin
36	TPM1	108	CD59	180	EphA3	252	He4	324	NAIP	396	PTN	468	Trappin-2
37	ALPP	109	CD71	181	EphA4	253	Hemopexin	325	Nanog	397	PTPRD	469	TRKB
38	Pro-MMP-13	110	CD74	182	EphA5	254	Hepcidin	326	NELL2	398	PYK2	470	Troponin I
39	AMICA	111	CD90	183	EphA6	255	HSP32	327	Nephrilysin	399	PYY	471	TYRO10
40	AMPKa1	112	CD97	184	EphA7	256	HOXA10	328	Galanin	400	Ras	472	TRPC1
41	Amylin	113	CD79 alpha	185	EphA8	257	Haptoglobin	329	Nesfatin	401	RBP4	473	TRPC6
42	ANGPTL3	114	CD200	186	EphB1	258	HSP10	330	Nestin	402	RECK	474	TRPM7
43	ANGPTL4	115	CEA	187	EphB2	259	HSP20	331	NET1	403	RELM alpha	475	Trypsin 1
44	Annexin A7	116	CEACAM-1	188	EphB3	260	HSP27	332	Netrin G2	404	Resistin	476	TSH
45	APC	117	Ceruloplasmin	189	EphB4	261	HSP40	333	Netrin-4	405	RET	477	TSLP
46	APCS	118	CFHR2	190	EphB6	262	HSP60	334	Neuropeptide Y	406	RIP1	478	TXK
47	Apelin	119	Chemerin	191	ERRa	263	HSP70	335	NF1	407	ROCK1	479	Uromodulin
48	Apex1	120	CHI3L1	192	Erythropoietin R	264	HSP90	336	NM23-H1/H2	408	ROCK2	480	TFF1
49	APN	121	Chromogranin A	193	ESAM	265	HSPA8	337	Presenilin 2	409	ROR1	481	VDUP-1
50	ApoA1	122	Chymase	194	EV15L	266	HTRA2	338	Notch-1	410	ROR2	482	VEGF R1
51	ApoA2	123	ciAP-2	195	EXTL2	267	IBSP	339	NPTX1	411	ROS	483	VGf
52	ApoA4	124	Ck beta 8-1	196	FABP1	268	IGF2BP1	340	NPTXR	412	RYK	484	VIPR2
53	ApoB	125	CKMB	197	FABP2	269	IGFBP-5	341	Progesterone	413	S100A4	485	VDR
54	ApoC2	126	Claudin-3	198	FABP4	270	IDUA	342	Ntn1	414	S100A6	486	VDB
55	ApoB100	127	Claudin-4	199	FAK	271	IL-33	343	OCT3/4	415	S100A8	487	PROS1
56	ApoE	128	CLEC3B	200	FAP	272	IL-34	344	Omentin	416	S-100b	488	Vitronectin
57	ApoE3	129	Clusterin	201	Fcg RIIB/C	273	INSRR	345	Osteocalcin	417	SART1	489	VWF
58	ApoD	130	CNDP1	202	Fen-1	274	ITGAV	346	Osteopontin	418	SART3	490	WT1
59	ApoM	131	Fc gamma RIIB	203	FER	275	CD61	347	OX40	419	SCG3	491	XIAP
60	ApoH	132	Factor XIII B	204	Ferritin	276	Itk	348	p21	420	Selenoprotein P	492	ZAG
61	APP	133	COCO	205	Fetuin A	277	ITM2B	349	p27	421	SEMA3A	493	ZAP70
62	ASPH	134	C2	206	Fetuin B	278	Kallikrein 2	350	p53	422	Serotonin		
63	Attractin	135	C3a	207	FGFR1	279	ApoC3	351	PAI-1	423	Serpin A1		
64	B3GNT1	136	C5a	208	FGFR1 alpha	280	Kallikrein 5	352	PAK7	424	Serpin A12		
65	BAF57	137	C7	209	FGFR2	281	Kallikrein 6	353	Pappalysin-1	425	Serpin A3		
66	BAFF	138	C8b	210	Fibrinogen	282	Kallikrein 7	354	PP	426	Serpin A4		
67	BAI-1	139	C9	211	Fibrinopeptide A	283	Kallikrein 8	355	Presenilin 1	427	Serpin A5		
68	BCAM	140	CFH	212	Fibronectin	284	Kallikrein 10	356	PARK7	428	Serpin A8		
69	B2M	141	Contactin-1	213	Ficolin-3	285	Kallikrein 11	357	Visfatin	429	Serpin A9		
70	Beta Defensin 4	142	Contactin-2	214	FIH	286	Kallikrein 14	358	P-Cadherin	430	Serpin B5		
71	Beta IG-H3	143	CBG	215	FOLR1	287	KCC3	359	PCAF	431	Serpin D1		
72	Biglycan	144	COX-2	216	FOXN3	288	KCTD10	360	PD-1	432	Serpin I1		

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	CUP170	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	ArgR5	157	Cadherin-6	229	CNOT1	301	DNER	373	fast skeletal Myosin	445	GRNPR
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	Guanylin
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 Ia1	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	ASH2L1	167	CapG	239	Collagen IVa6	311	DGK	383	Fibrinogen-like 2	455	HOGF
24	ADAS	96	ASGR2	168	CAPZA1	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	DNPPEP	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	DPP1	391	FKBP25	463	HGFA
32	AGA	104	ATPB	176	Cathepsin H	248	COP58	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	CCDC25	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	BCHC	186	CD16	258	CPS1	330	EFTUD2	402	G0/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	HUA-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	elF4A1	406	GAPDH	478	HMGB2
47	ALDH16A1	119	beta 1 Tubulin	191	CD41	263	CRMP2	335	elF5A	407	GARNL1	479	HMG83
48	ALDH1A1	120	CUBB3	192	CD42b	264	CRTAC1	336	ELAVL1	408	GART	480	HMG2
49	ALDH9A1	121	BID	193	CD48	265	CS	337	EMILUN1	409	Gastrokine 1	481	HN1
50	ALKP	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	GCDFP 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	CSR1	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 (n)	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	BTD	207	CFHR1	279	Cytokeratin 10	351	EPPK1	423	GLPR2	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	HRS12
67	Ankrd26	139	C1qB	211	CFL1	283	Cytokeratin 16	355	Erp29	427	GLUD1	499	HSC70
68	Annexin A1	140	C1qR1	212	CFV1	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	Chitobiase	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	Peroxioredoxin 3	289	PTK 7	361	Serpin A7	433	Thymosin b10
2	HTRA1	74	LMAN2	146	Nectin-3	218	Peroxioredoxin 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	PTPRZ	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	SH3BGR	446	TRF 2
15	ITGB6	87	MAGP-2	159	NIT2	231	plgR	303	RanGAP1	375	SH3BGR3	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 ICD	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	NTSC3	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	PLOD1	316	RNASE4	388	SLURP1	460	UBE2D3
29	KPNB1	101	MCMP2	173	OBCAM	245	PLOD2	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	Pldc2	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	Prosaposin	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	VSIG4
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	SAAM4a	419	Talin1	491	WISP2
60	LAMP	132	MYL12B	204	PCYOX1	276	Prouroguanylin	348	aAmylase	420	Talin1&2	492	WNK2
61	LAMP1	133	MYL3	205	PDE1B	277	PRSS23	349	SAMSN1	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	NAP111	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	SEMG1	430	TGM3		
71	SPINK5	143	NCAM2	215	Periostin	287	PSMD9	359	SEMG2	431	Thioredoxin-1		
72	LILRA3	144	Nebulin	216	Peroxioredoxin 2	288	PTEN	360	Serpin A11	432	THOP1		

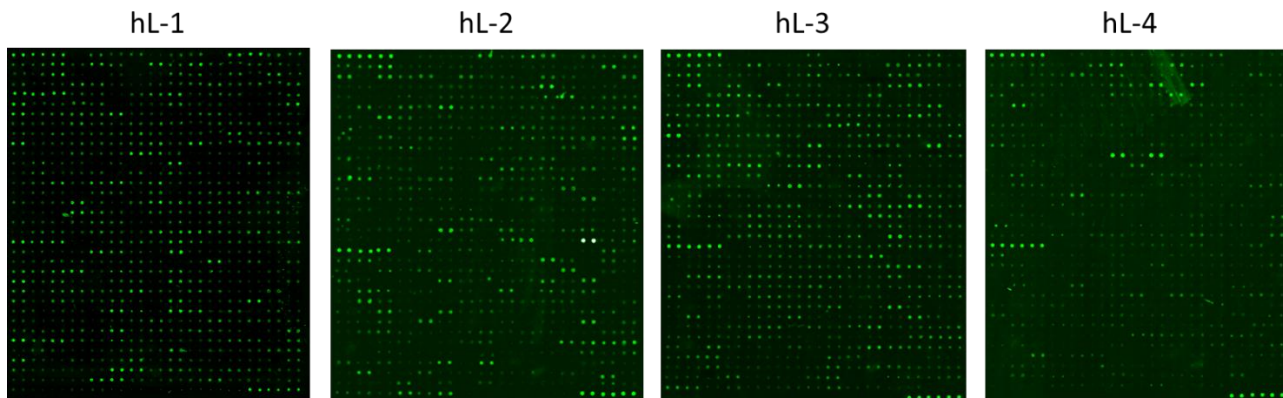
VII. Interpretation of Results:

A. Explanation of Controls Spots

There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized biotinylated IgG. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal responses to a known control. Some arrays may have beta-actin and GAPDH as internal controls, much as “housekeeping” genes or proteins are used to normalize results in PCR or Western blots, respectively.

B. Typical Results

The following figure shows the typical result of this array probed with sample(s). The images were captured using an Axon GenePix laser scanner. The Positive control signals in the upper left and lower right corners of each array can be used to identify the orientation and help normalize the results between arrays.



Note: In the absence of an external standard curve for each protein detected, there is no means of assessing absolute or relative concentrations of different proteins in the same sample using immunoassays. If you wish to obtain quantitative data (i.e., concentrations of the various analytes in your samples), try using our Quantibody® Arrays as a targeted follow-up experiment.

C. Background Subtraction

Once you have obtained fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Most laser fluorescence scanners' software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEAN signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "F532 Mean - B532"), you may need to subtract the background manually or change the default settings on your scanner's data report menu.

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 freely 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. Analysis Tool software can be downloaded from the product page on the RayBiotech website.

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 ≥ 1.5 -fold increase or ≤ 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

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Dilute starting sample less or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
General	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from drying out during experiment

IX. Selected References

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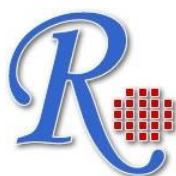
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