RayBio® Label-Based (L-Series) Human Antibody Array L-1000 Glass Slide Kit

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

Patent Pending Technology
User Manual (January 1, 2022)

For the simultaneous detection of the relative expression of 1000 human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Human Antibody Array L-1000
Cat# AAH-BLG-1000-4 (4 Sample Kit)
Cat# AAH-BLG-1000-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

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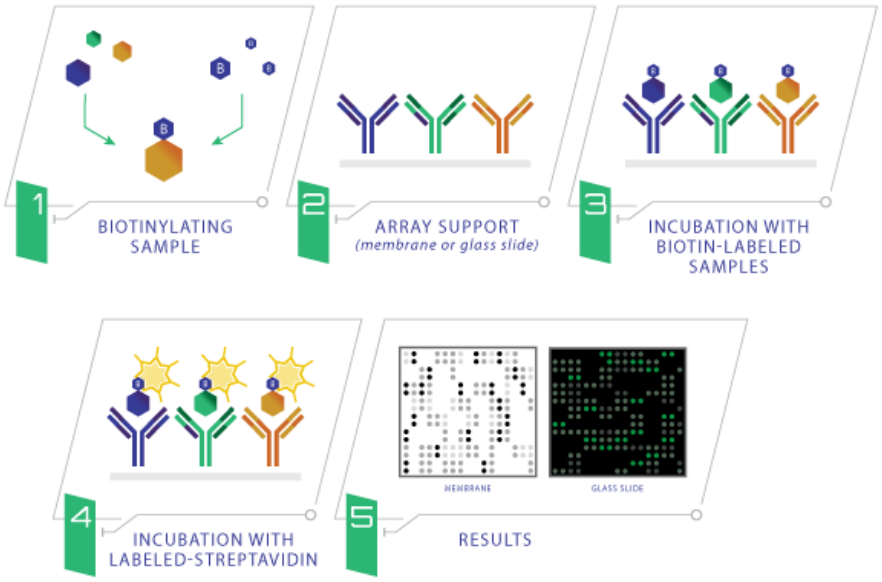
I. Introduction

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

*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 $1x10^6$ cells in 100 mm tissue culture dishes.*
   2. Culture cells in complete culture medium for ~24–48 hours.**
   3. Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours.**,† 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 ddH_2O). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.

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

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

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

3) Extracting Protein from Crude Tissue

1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O).

2. Homogenize the tissue according to homogenizer manufacturer instructions.

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

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

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

4) Determine the total protein concentration

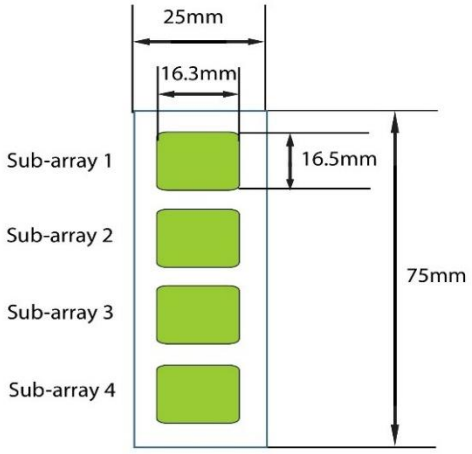
For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

B. Handling the 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

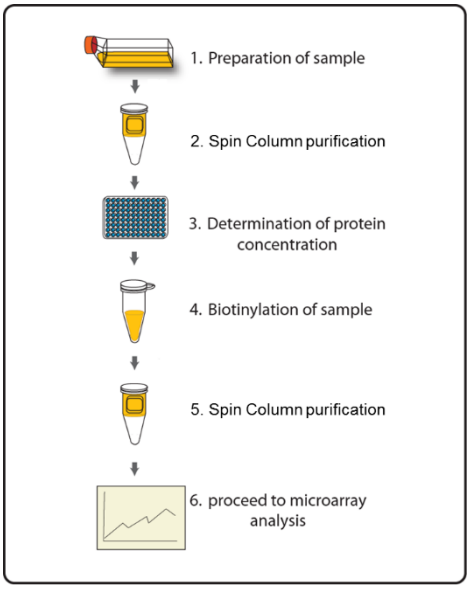
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.

- 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.

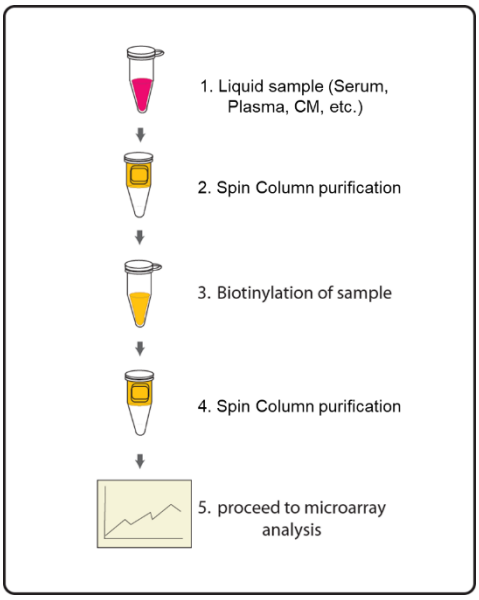
IV. Protocol

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 µl 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 µl neat supernatant*
    - *Serum/Plasma: 2 µl serum/plasma in 100 µl labeling buffer*
    - *Cell/tissue lysate: 20 µg lysate in 100 µl 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 µl for each Spin Column. Do not load over 130 µl of sample into a Spin Column.

B. Biotin-Labeling the Sample

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

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.

6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.

    a. For labeling cell culture supernatants: Add 8 µl of Labeling Reagent into the sample tube (for 120 µl supernatant).

b. For labeling serum or plasma: Add 8 µl of Labeling Reagent Solution into the sample tube (for 2 µl serum/plasma *in 100 µl labeling buffer*).

c. For labeling cell or tissue lysates: Add 4 µl of Labeling Reagent Solution into the sample tube (for 20 µg lysate *in 100 µl labeling buffer*).

d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

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

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

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

C. 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 µl 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 µl 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 ddH_2O.

14. Decant the samples from each well and wash 3 times with 800 µl 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 µl 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 µl of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800 µl of Blocking Buffer. Mix gently.

18. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400 µl of 1X Cy3-Conjugated Streptavidin to each sub-array. Cover the incubation chamber with the plastic adhesive strips.

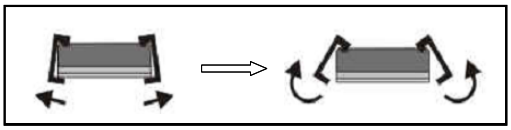
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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 ddH_2O 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.

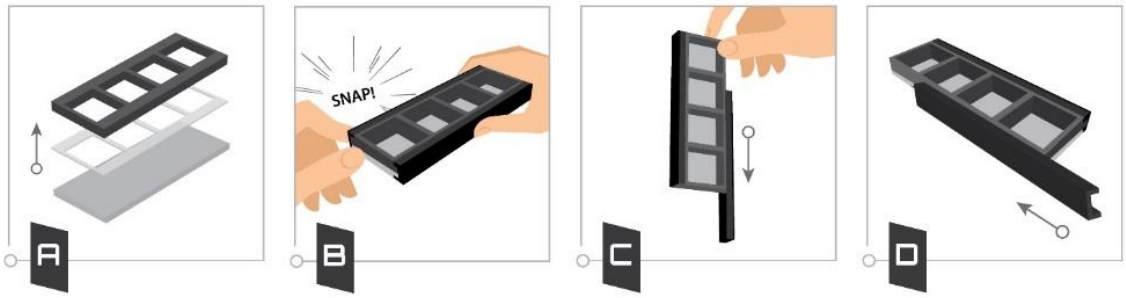
E. Fluorescence Detection

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: <u>Please protect the finished glass slides from temperatures above RT and store them in the dark</u>. 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

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B. RayBio® Human Antibody Array L-493 Array Map

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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	199	200	200	201	201	202	202	203	203	204	204	205	205	206	206
15	207	207	208	208	209	209	210	210	211	211	212	212	213	213	214	214	215	215	216	216	217	217	218	218	219	219	220	220	221	221
16	222	222	223	223	224	224	225	225	226	226	227	227	228	228	229	229	230	230	231	231	232	232	233	233	234	234	235	235	236	236
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
24	338	338	339	339	340	340	341	341	342	342	343	343	344	344	345	345	346	346	347	347	348	348	349	349	350	350	351	351	352	352
25	353	353	354	354	355	355	356	356	357	357	358	358	359	359	360	360	361	361	362	362	363	363	364	364	365	365	366	366	367	367
26	368	368	369	369	370	370	371	371	372	372	373	373	374	374	375	375	376	376	377	377	378	378	379	379	380	380	381	381	382	382
27	383	383	384	384	385	385	386	386	387	387	388	388	389	389	390	390	391	391	392	392	393	393	394	394	395	395	396	396	397	397
28	398	398	399	399	400	400	401	401	402	402	403	403	404	404	405	405	406	406	407	407	408	408	409	409	410	410	411	411	412	412
29	413	413	414	414	415	415	416	416	417	417	418	418	419	419	420	420	421	421	422	422	423	423	424	424	425	425	426	426	427	427
30	428	428	429	429	430	430	431	431	432	432	433	433	434	434	435	435	436	436	437	437	438	438	439	439	440	440	441	441	442	442
31	443	443	444	444	445	445	446	446	447	447	448	448	449	449	450	450	451	451	452	452	453	453	454	454	455	455	456	456	457	457
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	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	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	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	DEFA5
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	PlGF	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	Epiregulin	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	RELM 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	LIMPII	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	ADAMTSL2	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	TAF4
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	Neprilysin	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 RIIIB	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		

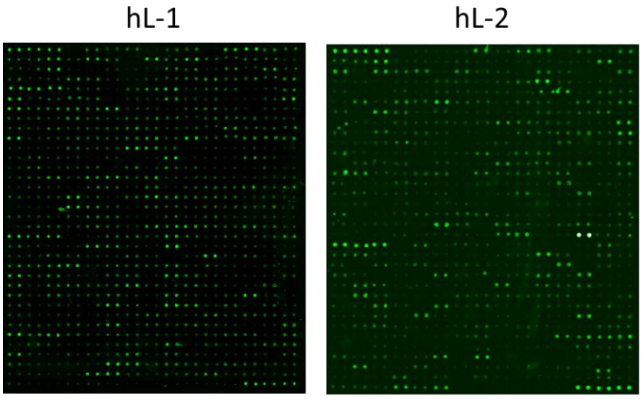
VII. Interpretation of Results:

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 ≈ 95%).

F. Pathway Analysis of the Array Proteins

Human antibody array L-1000 detects 1000 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 completed 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 dying out during experiment

IX. Selected References

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