RayBio® Label-Based (L-Series) Human Antibody Array L-3 or L-4

Patent Pending Technology
User Manual (Revised Dec 9, 2019)

For the simultaneous detection of the relative expression of 500 human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Human Antibody Array L-3
Cat# AAH-BLG-3-4 (4 Sample Kit)
Cat# AAH-BLG-3-8 (8 Sample Kit)

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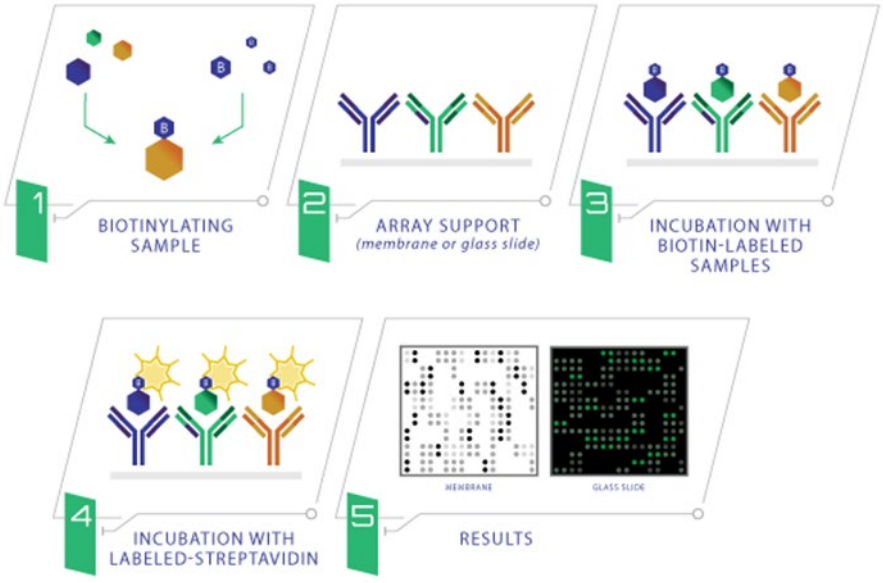
I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the L-Series Antibody Array L-3 or L-4, researchers can now obtain a broad, panoramic view of protein expression. The expression levels of 500 human target proteins can be simultaneously detected, including extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins in cell culture supernatants, cell lysate, tissue lysate, serum and plasma.

The first step in using the RayBio® L-Series Human Antibody Array L-3 or L-4 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. Use within 6 months from the date of shipment is recommended. 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 NOTE: all components (except slides) are identical between the L-3 and L-4 kits	AAH-BLG-3-4 (L-3) or AAH-BLG-4-4 (L-4)	AAH-BLG-3-8 (L-3) or AAH-BLG-4-8 (L-4)
A	Dialysis Vials & Floating Dialysis Rack	8 vials	16 vials
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio® L-Series Human Antibody Array L-3 or L-4 Glass Slides*	1 slide (L-3 or L-4)	2 slides (L-3 or L-4)
F	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml)
G	20X Wash Buffer I	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer II	1 bottle (30 ml)	1 bottle (30 ml)
I	Cy3-Conjugated Streptavidin	1 vial	2 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (8 ml)	
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	
M	30 ml Centrifuge Tube	1 tube	

*Each slide contains 2 identical subarrays

**Only needed if testing cell or tissue lysates

B. Additional Materials Required

- KCl, NaCl, KH_2PO_4, Na_2HPO_4 and ddH_2O
- 1 ml tube, small plastic or glass containers
- Orbital shaker or oscillating rocker
- Beaker, stir plate and stir bar
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection (list available online)
- 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 g for 10 min and store as ≤1 ml aliquots at -80°C until needed.
5. 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. Centrifuge 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. Proceed to b. Cells in Suspension.

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

2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2 fold with ddH_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 min at 2-8 °C.

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

4. Transfer lysates to a clean tube. 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 min at 13,000 rpm (4°C).

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

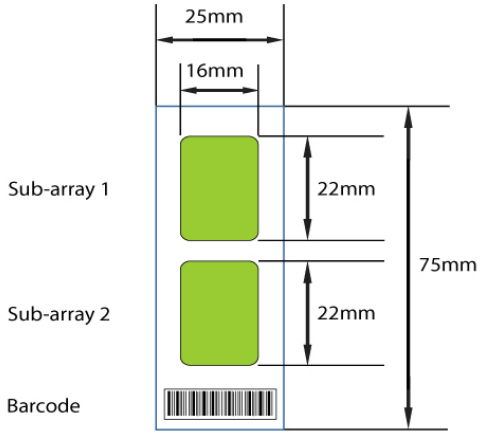
4. Transfer supernatant to a clean tube and store at -80°C.

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 on page 13, and take great care not to break the glass slide when doing so.
- Permanent marker ink can significantly interfere with fluorescent signal detection. Never mark anywhere on the front (arrayed) side of the slide. It's best to avoid using marker completely, however if you need to number the slide, please add a small mark only on the back of the slide along the top or bottom edge using a green or blue ultra-fine point Sharpie® brand marker, only after the slide is completely dry.
- 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 Human L-3 and L-4 Glass Slide

Two identical sub-arrays on one slide



2 printed sub-arrays per glass chip

D. Incubations and Washes

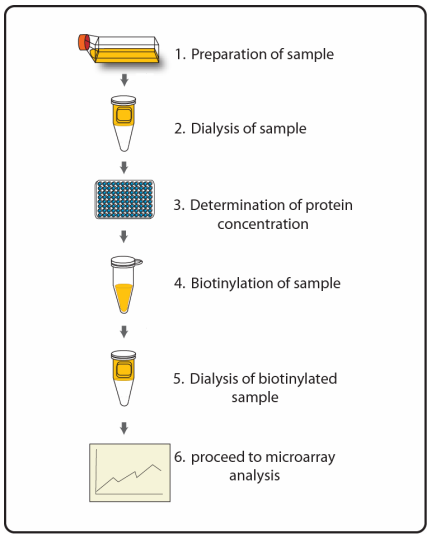
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.
- Perform all incubation and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

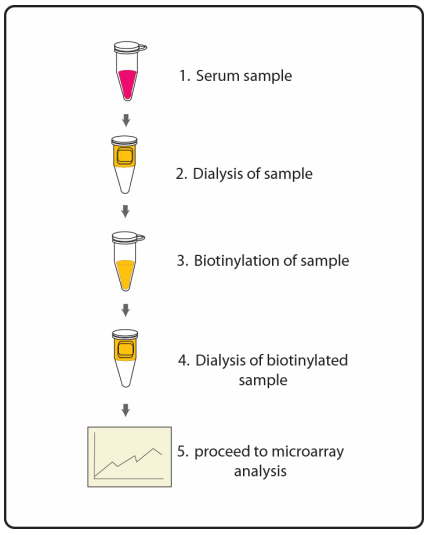
IV. Protocol

Assay Diagram

1. Cell culture supernatants or cell/tissue lysates



2. Serum or plasma



Note: If using cell or tissue lysates, start at "Dialysis of sample"

A. Dialysis of Sample

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

1. Prepare enough dialysis buffer (1X PBS, pH=8.0) for all dialysis steps herein and after. To prepare 1 L dialysis buffer, dissolve 0.2 g KCl, 8 g

NaCl, 0.2 g KH_2PO_4 and 1.15 g Na_2HPO_4 in 800 ml ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 ml with ddH_2O.

2. Add each sample into a separate Dialysis Tube (Item A). Loading volumes are as follows: 200 µl cell culture supernatant; 100 µl cell or tissue lysate (1~2 mg/ml total protein); 20 µl serum or plasma + 80 µl dialysis buffer (5-fold dilution). Carefully place Dialysis Tubes into Floating Dialysis Rack.

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

3. Place Floating Dialysis Rack into ≥500 ml dialysis buffer in a large beaker. For more than 2 samples, make certain to use at least 300 ml dialysis buffer for each sample (more buffer will improve the efficiency of dialysis). Place beaker on a stir plate and dialyze, for at least 3 hours at 4˚C, stirring buffer gently. Then exchange the dialysis buffer and repeat dialysis for at least 3 hours at 4˚C. Transfer dialyzed sample to a clean microfuge tube. Spin dialyzed samples for 5 min at 10,000 rpm to remove any particulates or precipitates, and then transfer the supernatants to a clean tube.

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

Note: Determine the total protein concentration for cell culture supernatants or cell/tissue lysate after dialysis procedure (Step 3). We recommended using a BCA total protein assay (eg, Pierce, Catalog # 23227).

B. Biotin-labeling Sample

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

4. Immediately before use, prepare 1X Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl 1X PBS into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.

5. Add 1X Labeling Reagent to dialyzed samples.

    a. For labeling cell culture supernatants: transfer 180 µl dialyzed sample into a new tube. Add 36 µl of 1X Labeling Reagent Solution per 1 mg total protein in dialyzed cell culture supernatant. Mix well. For example, if sample's total protein concentration is 0.5 mg/ml you need to add 3.24 µl 1X Labeling Reagent to the tube of 180 µl dialyzed sample.

    b. For labeling serum or plasma: Add 22 µl of 1X Labeling Reagent Solution into a new tube containing 35 µl dialyzed serum or plasma sample and 155 µl Labeling Buffer (Item K).

    c. For labeling cell or tissue lysates: transfer 30 µg (15 µl of 2 mg/ml) cell or tissue lysates into a tube and add labeling buffer (Item K) for a total volume of 260 µl. Then add 3.3 µl of 1X Labeling Reagent Solution.

Note: To normalize serum/plasma or cell/tissue lysate concentrations during biotinylation, measure sample volume before and after dialysis. Then adjust the volumes of dialyzed serum/plasma or cell/tissue lysates and Labeling Buffer to compensate. For example, if the sample volume doubles after dialysis, then use twice as much serum/plasma in the labeling reaction (70 µl) and reduce the Labeling Buffer to 120 µl.

6. 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.

7. Add 3 µl Stop Solution (Item D) into each reaction tube. Collect and transfer each sample from reaction tube into a separate Dialysis Tube (Item A). Immediately dialyse samples as directed in Step 3 on pages 9.

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 approx. 15 min, 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 min. Ensure there are no bubbles on the array surfaces.
11. Immediately prior to sample incubation, spin biotin-labeled samples for 5 min at 10,000 rpm to remove any particulates or precipitates. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 2-10 folds for cell culture supernatants, 20 folds for serum/plasma and 30 folds for cell/tissue lysate.

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 Blocking Buffer from each well. Add 400 µl of diluted samples 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 min 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 min 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 min 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 tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do <u>not</u> 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.

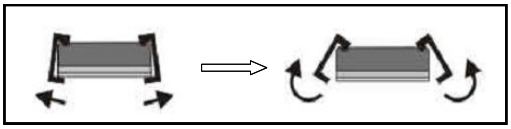
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 2 hours 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. Repeat step 20, this time with 1X Wash Buffer II. Repeat one time for a total of two washes for 5 min per wash.

23. Finally, wash the glass slide with 30 ml of ddH_2O for 5 min. 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 slides in a laminar flow hood for 20 minutes or until slide is completely dry.
- 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, only the sides.

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

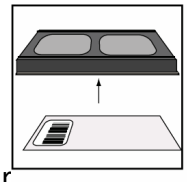
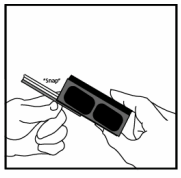
E. Fluorescence Detection

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)*

A   B

C D

V. Antibody Array Maps and Target Lists

A. RayBio® Human Antibody Array L-3 and L-4 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	P 1a	P 1a	P 2a	P 2a	P 3a	P 3a	neg	neg	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12	13	13	14	14	15	15
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26	376	376	377	377	378	378	379	379	380	380	381	381	382	382	383	383	384	384	385	385	386	386	387	387	388	388	389	389	390	390
27	391	391	392	392	393	393	394	394	395	395	396	396	397	397	398	398	399	399	400	400	401	401	402	402	403	403	404	404	405	405
28	406	406	407	407	408	408	409	409	410	410	411	411	412	412	413	413	414	414	415	415	416	416	417	417	418	418	419	419	420	420
29	421	421	422	422	423	423	424	424	425	425	426	426	427	427	428	428	429	429	430	430	431	431	432	432	433	433	434	434	435	435
30	436	436	437	437	438	438	439	439	440	440	441	441	442	442	443	443	444	444	445	445	446	446	447	447	448	448	449	449	450	450
31	451	451	452	452	453	453	454	454	455	455	456	456	457	457	458	458	459	459	460	460	461	461	462	462	463	463	464	464	465	465
32	466	466	467	467	468	468	469	469	470	470	471	471	472	472	473	473	474	474	475	475	476	476	477	477	478	478	479	479	480	480
33	481	481	482	482	483	483	484	484	485	485	486	486	487	487	488	488	489	489	490	490	491	491	492	492	493	493	494	494	495	495
34	496	496	497	497	498	498	499	499	500	500	501	501	502	502	503	503	504	504	505	505	506	506	507	507	508	508	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	positi	posit	posit	posit	posit	positi

B. RayBio Human Antibody Array L-3 Target List

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

RayBio Human Antibody Array L-3 Target List… continued

number	name	number	name	number	name	number	name
301	DARS2	361	ERAB	421	GCLC	481	Histone H2B K
302	DCI	362	ERAP2	422	GCSH	482	Histone H3.3
303	DCXR	363	ERp29	423	GDA	483	Histone H4
304	DDAH1	364	ERp57	424	GDF7	484	HLA-C
305	DDT	365	ERp72	425	GDI1	485	HMGB1
306	DDX3Y	366	ESD	426	GDI2	486	HMGB2
307	DEFA6	367	ESR1	427	Gephyrin	487	HMGB3
308	DEP-1	368	ETL	428	GFAP	488	HMGN2
309	Der p2	369	EVC2	429	GHRF	489	HN1
310	Dermcidin	370	Ezrin	430	GIP	490	HNF-3 alpha /FoxA1
311	Desmocollin 1	371	F11	431	GLIPR2	491	hnRNP A1
312	Desmocollin-2	372	FABP5	432	GLRX1	492	hnRNP A2B1
313	Desmocollin-3	373	Factor IX	433	G6PD	493	hnRNP C1 + C2
314	Desmoglein-1	374	Factor V	434	Glucosidase 2 subunit beta/PRKCSH	494	hnRNP G
315	Desmoglein-2	375	Factor XII	435	GLUD1	495	hnRNP L
316	Desmoplakin	376	Factor XIII	436	Glutamyl hydrolase gamma /CGH	496	hnRNP M1-M4
317	Desmuslin	377	FAM20C	437	glutathione S transferase Omega 1/GSTO1	497	hnRNP U
318	Destrin	378	FAM3C	438	Glutathione Synthetase/GSS	498	Hornerin
319	DGK	379	Fascin	439	Glycerol 3 Phosphate Dehydrogenase	499	Hoxb3
320	DISC 1	380	FASN	440	Glycoprotein V	500	HOXD11
321	DMGDH	381	fast skeletal Myosin	441	Glyoxalase II	501	HP1BP3
322	DMRN9	382	FASTKD5	442	GM2A	502	HPD
323	Dopamine beta Hydroxylase/DBH	383	FBP 38	443	GMF beta	503	HPR
324	DOT1L	384	FBP2	444	GNB1	504	HPRT
325	DPEP2	385	FBPase 1	445	GNPTG	505	HRG
326	DPP3	386	FCGBP	446	GOLPH2	506	HRSP12
327	DPPI	387	FDPS	447	GOLPH4	507	HSC70
328	DRIL1	388	FH	448	GOT2	508	HSP47
329	DSCAM	389	Fibrillin 1	449	GPCR GPR116	509	Neg
330	DSPG3	390	Fibrinogen gamma chain/FGG	450	GPLD1	510	Neg
331	Dystroglycan	391	Fibrinogen-like 2	451	Grainyhead-like protein 1 homolog/GRHL1	511	Neg
332	E1 Ubiquitin Activating Enzyme/UBA1	392	Fibrinopeptide B	452	Granzyme M	512	Neg
333	ECHS1	393	Fibulin 3	453	GRHPR	513	Neg
334	ECM-1	394	Ficolin-2	454	GRP	514	Neg
335	EEF1G	395	Filamin A	455	GSTM1	515	Neg
336	EEF2	396	Filamin B	456	GSTP1	516	Neg
337	EFEMP2	397	Filamin C	457	Guanylin	517	Neg
338	EFTUD2	398	FKBP12	458	GULP1/CED-6	518	Neg
339	EHD1	399	FKBP25	459	H6PD	519	Neg
340	EHD3	400	FKBP51	460	HABP2	520	Neg
341	EIF3S2	401	FLG2	461	HBZ	521	Neg
342	eIF4A1-N-t	402	FOLR3	462	HCFC1	522	Neg
343	eIF5A	403	Frizzled 8	463	HDGF	523	Pos 1c
344	ELAVL1	404	FRY	464	HEG1	524	Pos 2c
345	EMILIN1	405	FSH	465	Hemoglobin	525	Pos 3c
346	EMSY	406	FTL	466	Hemoglobin A1c	526	
347	EN2	407	FUCA1	467	Hemoglobin subunit beta/HBB	527	
348	Endorepellin	408	FUCA2	468	Hemoglobin subunit delta/HBD	528	
349	ENO1	409	Fumarylacetoacetate hydrolase/FAH	469	Hemoglobin subunit gamma 2/HBG2	529	
350	ENO1 + ENO2 + ENO3	410	G0/G1switch 2	470	HEXB	530	
351	ENSA	411	G3BP	471	HGFA	531	
352	Envoplakin	412	GALNT2	472	hGH	532	
353	Eosinophil derived neurotoxin/EDN	413	gamma Catenin	473	hHR23b	533	
354	EPB41	414	GAPDH	474	HIBADH	534	
355	EPCR	415	GARNL1	475	HINT1	535	
356	Ephrin B1	416	GART	476	HIP1R	536	
357	Ephrin B2	417	Gastrokine 1	477	Histone H1.2	537	
358	EPHX2	418	GATM - C-terminal	478	Histone H1.3	538	
359	EPPK1	419	GBE1	479	Histone H2A	539	
360	Eps 15	420	GCDFP 15	480	Histone H2A.Z	540	

C. RayBio Human Antibody Array L-4 Target List

number	name	number	name	number	name	number	name	number	name
1	Pos 1a	61	Laminin 2 alpha	121	MN1	181	Orosomucoid 2	241	Plakophilin 1
2	Pos 2a	62	Laminin b2	122	Moesin	182	ORP150	242	Plastin L
3	Pos 3a	63	Laminin gamma 1	123	MP1	183	OSBP1	243	PLC-gamma 1
4	Neg	64	LAMP	124	MPCA	184	OSCAR	244	Pleckstrin
5	HSPA1A	65	LAMP1	125	MPO	185	OSM R beta	245	Plectin
6	HTRA1	66	LAMP2	126	MRP 1	186	Osteoadherin(2)	246	Plexin B1
7	Human Agrin	67	LAP3	127	MSH6	187	Oxytocin-neurophysin 1/OXT	247	Plexin B2
8	IBP160	68	LASP1	128	mTOR	188	p16 ARC	248	PLOD1
9	IDH1	69	Latent TGF beta bp2	129	MUCDHL	189	P20Sb3	249	PLOD2
10	IDH3A	70	LCAT	130	Multimerin 2	190	p23	250	PLS3
11	IFRD1	71	LCMT2	131	MyBPC3	191	p39	251	Plxdc2
12	IGF2BP2	72	LDHA	132	MYH2	192	P4HB	252	PNP
13	IGFBP7	73	LDHB	133	MYH6	193	p73	253	POR
14	IGSF4B	74	LEDGF	134	MYH7	194	PA2G4	254	PPCS
15	Ihh	75	LEKTI/SPINK5	135	MYHC	195	PABP	255	PPOX
16	ILK	76	LILRA3	136	MYL12B	196	PACS1	256	PPP2R1B
17	Inhibin beta	77	LIMS1	137	MYL3	197	PARVB	257	PPP2R4
18	Integrin b1	78	LMAN2	138	MYO5A	198	PCBP1	258	PRCP
19	Integrin beta 6	79	LMW-PTP/ACP1	139	Myoferlin	199	PCBP2	259	PRDM13
20	Intergrin a6	80	LOK	140	Myosin 18B	200	PCCA	260	PRDX 1
21	IQGAP1	81	LOX	141	Myotrophin	201	PCDH7	261	PRELP
22	IQGAP2	82	LOXL1	142	NABC1	202	PCDX8	262	PREP
23	IRE1	83	LRP 4	143	NAGLU	203	PCK2	263	PRG2
24	IRS2	84	LTA4H	144	NAP1L1	204	PCMT1	264	Prion protein PrP /PRNP
25	ISOC2	85	LTBP4	145	NAPRT1	205	PCNA	265	Profilin 1
26	ITGB4BP	86	Lubricin	146	NASP	206	PCPE-1	266	Properdin
27	ITIH1	87	LUZP1	147	NCAM2	207	PCSK9	267	Prosaposin
28	ITIH2	88	LYPA1	148	Nebulin	208	PCYOX1	268	Prostaglandin D Synthase/PTGDS
29	ITIH3	89	Lysozyme	149	Nectin-1	209	PDE1B	269	Proteasome 20S a+b
30	ITIH4 a	90	MAGI2	150	Nectin-3	210	PDIA6	270	Proteasome 20S alpha
31	JAM-A	91	MAGP-2	151	NEDD8	211	PDLIM1	271	Proteasome 20S alpha 5
32	JARID2	92	MAN1	152	Neogenin	212	PDLIM5	272	Proteasome 20S b7
33	Karyopherin beta 1	93	MANF	153	Nesprin2	213	PDZD2	273	Proteasome 26S S5
34	Keratin 36	94	Mannosidase II	154	Neurabin 1	214	PEBP4	274	Proteasome beta 1
35	Keratin 38	95	MAP1A	155	Neural Cadherin	215	PEPD	275	Proteasome subunit alpha 6/PSMA6
36	KHSRP	96	MAPRE1	156	Neurofibromin	216	PER1	276	Proteasome subunit beta 2/PSB2
37	KIAA0319L	97	MARCKS	157	Neurogranin	217	perilipin 3	277	Proteasome subunit beta 4/PSB4
38	KIAA1468	98	MASP3	158	Neuropeptide B	218	Perilipin-1	278	Protein C
39	KIAA1967	99	MBD2	159	Neuropilin-1	219	Periostin	279	Protein Z
40	KIF5B	100	MBP	160	Neurotrimin	220	Peroxiredoxin 2	280	Prouroguanylin
41	Kilon	101	MCAM	161	NF-M	221	Peroxiredoxin 3	281	PRSS23
42	KLK-B1	102	Mcl-1	162	Nidogen-2	222	Peroxiredoxin 5	282	PRSS3
43	KMD4B	103	MCM	163	NIT2	223	PF4V1	283	PRTN3
44	KMT2B	104	MCM5	164	NME3	224	PGAM1	284	PSMA1
45	KRT31	105	MCMP2	165	nNOS	225	PGAM2	285	PSMA2
46	KRT72	106	MDH1	166	Noelin	226	PGD	286	Pos 1b
47	Krt73	107	MDH2	167	Non-muscle Actin/Actin	227	PGDF/PHGDH	287	Pos 2b
48	KRT82	108	ME1	168	non-muscle Myosin IIA/Myosin	228	PGK-1	288	Pos 3b
49	KRT85 - N-terminal	109	MEP1A	169	Notch-2	229	PGLS-C-t	289	Neg
50	KRTDAP	110	Metallothionein	170	Notch-2 ICD	230	PGM1	290	PSMA4
51	KRTHA3B	111	Metavinculin	171	NPAS3	231	PGRPL	291	PSMA7
52	KSR1	112	MFAP4	172	NPM1	232	PHAP1	292	PSMB5
53	LAD	113	MFI2	173	NQO2	233	PSAT1	293	PSMC3
54	LAF4	114	mGLUR5	174	NT5C3	234	PI 3-Kinase C2 beta	294	PSMD1
55	LAIR1	115	MGP	175	Nucleobindin 1/NUCB1	235	pIgR	295	PSMD9
56	LAM b1	116	Mimecan	176	NUP98	236	PIK3IP1	296	PTEN
57	LAMA	117	MINPP1	177	OBCAM	237	PIN	297	PTK 7
58	Lamin A + C	118	MLCK	178	OIT3	238	PISD	298	PTMA
59	Lamin B1	119	MMR	179	Olfactomedin-2	239	PKLR	299	PTP gamma
60	Lamin B2	120	MMRN1	180	OTC	240	PLA2G1B	300	PTP kappa

RayBio® Human Antibody Array L-4 Target List ...continued

number	name	number	name	number	name	number	name
301	PTP mu	361	SDNSF	421	SUMO3	481	URB
302	PTPRS	362	SDPR	422	Symplekin	482	URB2
303	PTPRZ	363	Secretogranin V/SCG5	423	SynCAM	483	UROC1
304	PYGL	364	Semaphorin 6B	424	Syntaxin 7	484	UROD
305	PZP	365	Semaphorin 7A	425	TAB182	485	URP2
306	QDPR	366	Semenogelin I/SEMG1	426	TAGLN2	486	USP14
307	QPRT	367	Semenogelin II/SEMG2	427	Talin1	487	USP5
308	Quiescin Q6	368	Serpin A11	428	Talin1&2	488	Uteroglobin(1)
309	Rab7a	369	Serpin A7	429	TAX1BP3	489	Utrophin
310	Ran	370	Serpin B3/SCCA1	430	TBCA	490	VAP-1
311	RanGAP1	371	Serpin B6	431	TCEB2	491	VAP-A
312	RAP1AB	372	Serpin B8	432	Tcf20	492	VCP
313	Rbm15	373	Serpin F2	433	TCN1	493	VDAC1 / Porin
314	RCL	374	Serpin A10/ZPI	434	TCP1 eta	494	Versican isoform V0
315	Reg1A	375	SERPINB1	435	Tenascin C	495	Vimentin B
316	Reg3A	376	SerpinB4	436	Tenascin X(1)	496	VNN1
317	RHOC	377	SerpinE2	437	TFF2(1)	497	VSIG4
318	RhoGDI	378	SerRS	438	TGM3	498	WDR1
319	Ribonuclease A	379	SET	439	Thioredoxin-1	499	WISP2
320	Ribonuclease Inhibitor	380	SEZ6L2	440	THOP1	500	WNK2
321	Ribonuclease T2	381	SF20	441	Thymosin b10	501	YB1
322	RKIP	382	SH3BGRL	442	Titin	502	YY1
323	RNA Polymerase II/POLR2A	383	SH3BGRL3	443	TLS/FUS	503	ZBTB4
324	RNASE4	384	SHANK1	444	TMEM223	504	ZC3H4-N-t
325	RNASE6	385	SHC1	445	TOB2	505	ZC3H8
326	RPL10	386	SHIP	446	TOP2B	506	ZDHHC18
327	RPL10A	387	SHMT1	447	TPM4	507	ZNF671
328	RPL11	388	SHP-1	448	TPP1	508	Zyxin
329	RPL12	389	Siglec-1	449	Transaldolase 1/TALDO1	509	Neg
330	RPL14	390	SIGLEC14	450	Transketolase/TALDO	510	Neg
331	RPL17	391	SIM2	451	Transthyretin	511	Neg
332	RPL22	392	SIRP beta 1/CD172b	452	TRAP1	512	Neg
333	RPL5	393	Six3	453	TRAP220	513	Neg
334	RPL7A	394	SLC38A10	454	TRF 2	514	Neg
335	RPLP0	395	SLITRK1	455	Triosephosphate isomerase/TPIS	515	Neg
336	RPS10	396	SLURP1	456	Tropomyosin 3	516	Neg
337	RPS11	397	SMA	457	TRP-1	517	Neg
338	RPS12	398	SMC4	458	TRPS1	518	Neg
339	RPS19	399	SMPD4	459	Trypsinogeb-2	519	Neg
340	RPS2	400	SOD1	460	TrypsinPan	520	Neg
341	RPS20	401	SOD2	461	Tryptophanyl	521	Neg
342	RPS23	402	SOD-3	462	TSR2	522	Neg
343	RPS25	403	SOD4	463	TUBA6	523	Pos 1c
344	RPS28	404	Somatostatin	464	TWF2	524	Pos 2c
345	RPS3	405	SORD	465	TXNDC4	525	Pos 3c
346	RPS5	406	SorLA	466	TXNDC5	526	
347	RREB1	407	SOX4	467	TXNRD2	527	
348	RSU1	408	SP-D	468	UBE2D3	528	
349	S100A1	409	Spectrin beta-5	469	Ube2L3	529	
350	S100A11	410	SPEN	470	UBE2N/Ubc13	530	
351	S100A7	411	SPINK7	471	Ubiquitin	531	
352	S100A9	412	SPTBN1	472	UCH-L1	532	
353	S100P	413	Src(1)	473	UFM 1	533	
354	SAA	414	SREC-II	474	UGGT	534	
355	SAA4a	415	STAT3	475	UNC-13 Homolog D	535	
356	Salivary alpha amylase/aAmylase	416	Stathmin 1	476	UNC45A	536	
357	SAMSN1	417	Sterol carrier protein 2/SCP2	477	UNC5H4	537	
358	SBP-1	418	STI1	478	UPB1	538	
359	SBSN	419	STOM	479	UQCRB	539	
360	SDF4	420	SUCLG1	480	UQCRH	540	

VI. Interpretation of Results:

A. Explanation of Controls Spots

1) Positive Control spots (POS1, POS2, POS3) are standardized amounts of biotinylated IgGs printed directly onto the array. 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, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

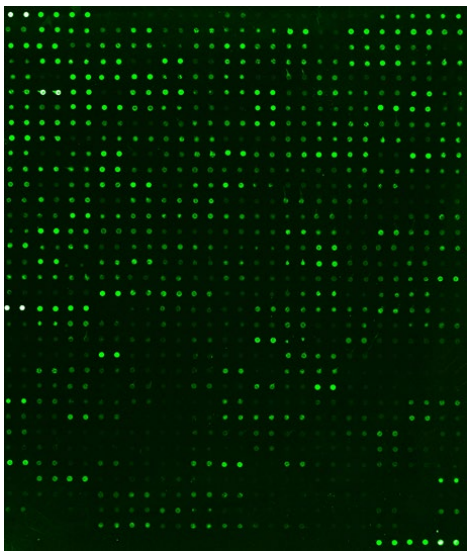
2) Negative Control (NEG) spots contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical Results

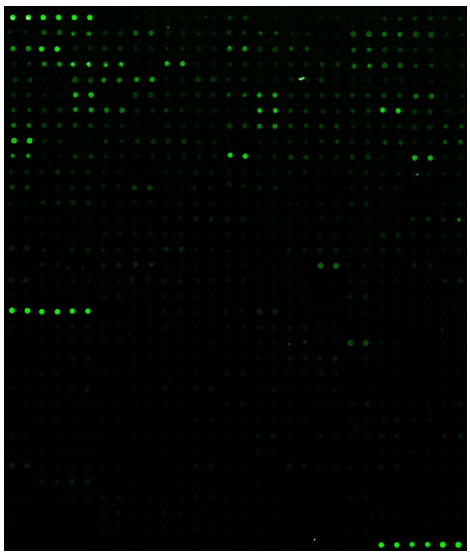
The following figure shows the RayBio® L-Series Human Antibody Array 1000 probed with serum sample. The images were captured using a Axon GenePix laser scanner. The strong signals in row 20 and the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays.

RayBio® L-Series Human Antibody Array L-3

Sample-1

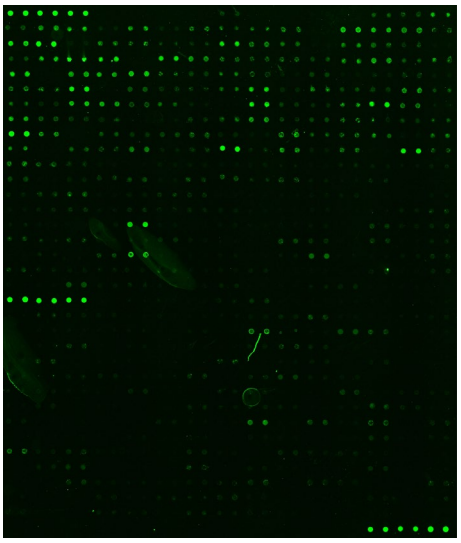


Sample-2

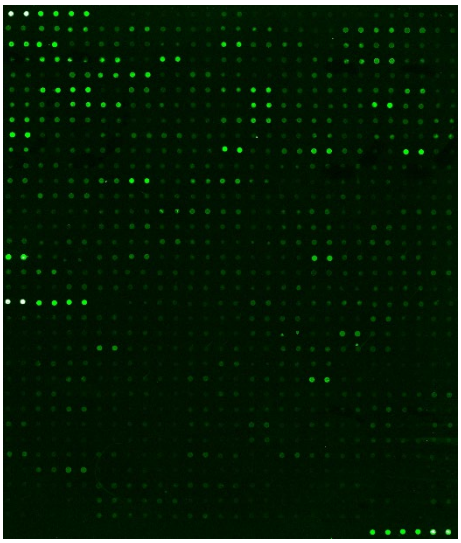


RayBio® L-Series Human Antibody Array L-4

Sample-1



Sample-2



If scanned using optimal settings, 3 distinct signal intensities will be seen: POS1>POS2>POS3. If all of these signals are of similar intensity, try increasing or decreasing laser power and/or signal gain settings.

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

C. Background Subtraction

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

Most laser fluorescence scanners' software have an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEDIAN background signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "MED532-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 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.

To order the Analysis Tool, please contact us at +1-770-729-2992 or info@raybiotech.com for more information.

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%).

VII. 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

VIII. Selected References

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