RayBio® Label-based (L-Series) Human Antibody Array 1000

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

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

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.

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

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

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

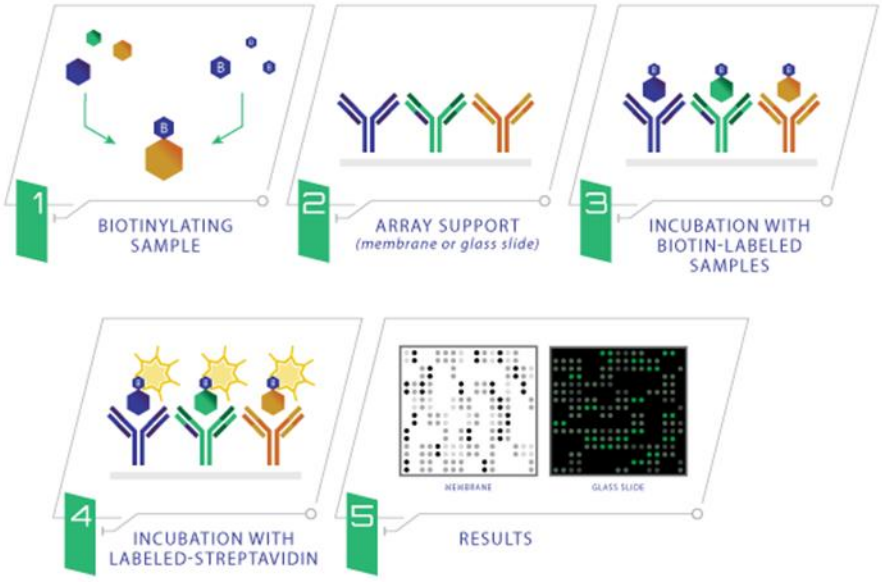
Tel: (Toll Free) 1-888-494-8555 or +1-770-729-2992; Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

TABLE OF CONTENTS

I. Introduction

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

The first step in using the RayBio® L-Series Human Antibody Array 1000 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 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	Cat# AAH-BLG-1000-4	Cat# AAH-BLG-1000-8
A	Dialysis Vials & Floating Dialysis Rack	8 vials	16 vials
B	Labeling Reagent	2 vials	4 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio® L-Series Human Antibody Array L-1000 Glass Slides*	1 L-507 slide	2 L-507 slides
		1 L-493 slide	2 L-493 slides
F	Blocking Buffer	2 bottles (8 ml/ea)	4 bottles (8 ml/ea)
G	20X Wash Buffer I	1 bottle (30 ml)	2 bottles (30 ml/ea)
H	20X Wash Buffer II	1 bottle (30 ml)	2 bottles (30 ml/ea)
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 4 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.

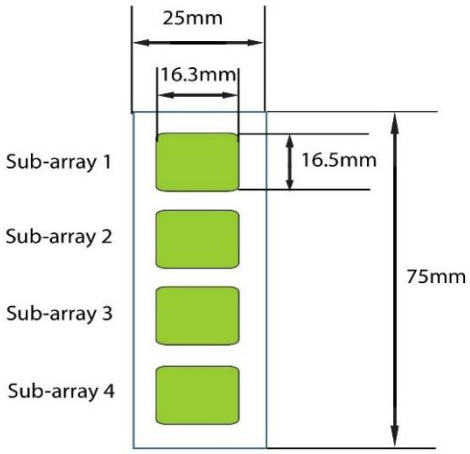
4. Transfer supernatant to a clean tube and store at -80°C.

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 14, 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-507 and L-493 Glass Slide

4 identical sub-arrays on one slide



4 printed sub-arrays per glass chip

D. Incubations and Washes

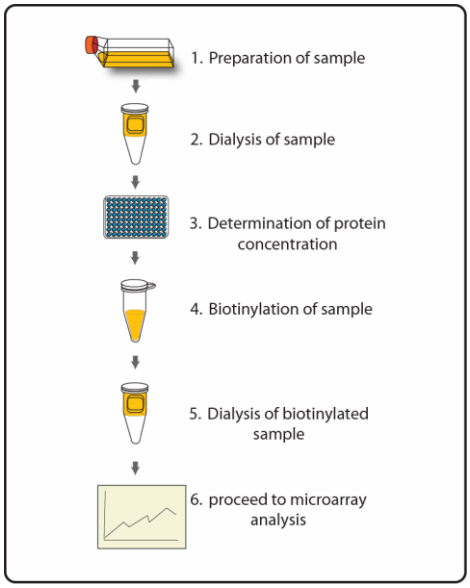
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.
- Perform all incubation and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

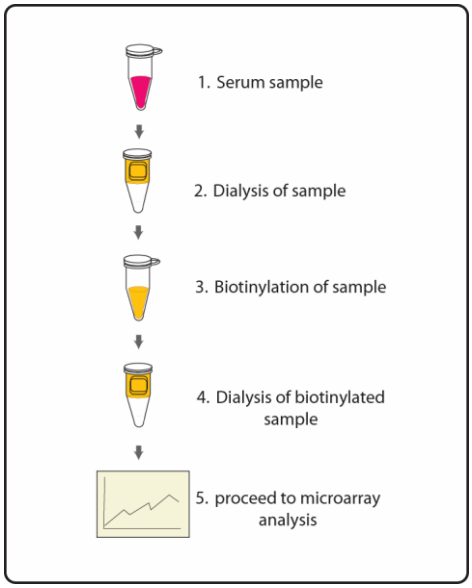
IV. Protocol

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. To prepare dialysis buffer (1X PBS, pH=8.0), dissolve 0.6 g KCl, 24 g NaCl, 0.6 g KH_2PO_4 and 3.45 g Na_2HPO_4 in 2500 ml ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 3000 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 for cell culture supernatants: if using a 2-fold dilution of biotin-labeled sample in the array incubation step (page 11, step 11), you will need to load a total of 400 µl of original cell culture supernatant into 2 separate Dialysis Tubes (200 µl /tube).

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. 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 minutes 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.

Note: You need to biotin-label 360 µl of dialyzed sample if dilution of the biotin-labeled samples is 2 fold in step 11 on page 11.

    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 (e.g. 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 concentrations during biotinylation, measure sample volume before and after dialysis. Then adjust the volumes of dialyzed serum/plasma 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 minutes. 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 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 <u>not</u> 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 <u>completely</u> 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. Immediately prior to sample incubation, spin biotin-labeled samples for 5 minutes 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 fold for cell culture supernatants, 20-fold for serum/plasma and 30 fold 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 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.

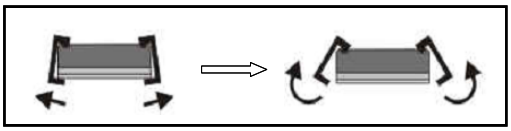
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 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.

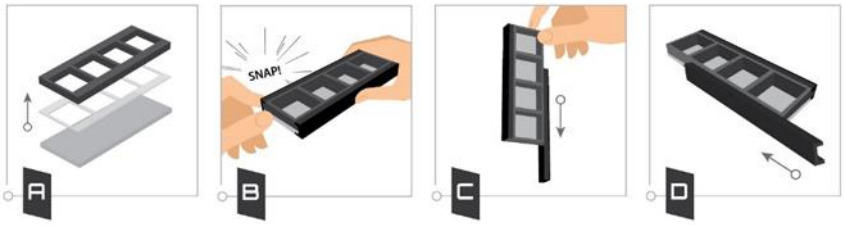
E. Fluorescence Detection

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

Note: Unlike most Cy3 fluors, the Streptavidin-Conjugated Fluor*used in this kit is very stable at RT and resistant to photobleaching on completed glass slides. <u>However, please protect 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 Maps and Target Lists

A. RayBio® Human Antibody Array L-507 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
2	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26	27	27	28	28	29	29	30	30
3	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41	42	42	43	43	44	44	45	45
4	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60
5	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75
6	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86	87	87	88	88	89	89	90	90
7	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101	102	102	103	103	104	104	105	105
8	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116	117	117	118	118	119	119	120	120
9	121	121	122	122	123	123	124	124	125	125	126	126	127	127	128	128	129	129	130	130	131	131	132	132	133	133	134	134	135	135
10	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146	147	147	148	148	149	149	150	150
11	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160	161	161	162	162	163	163	164	164	165	165
12	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176	177	177	178	178	179	179	180	180
13	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191	192	192	193	193	194	194	195	195
14	196	196	197	197	198	198	199	199	200	200	201	201	202	202	203	203	204	204	205	205	206	206	207	207	208	208	209	209	210	210
15	211	211	212	212	213	213	214	214	215	215	216	216	217	217	218	218	219	219	220	220	221	221	222	222	223	223	224	224	225	225
16	226	226	227	227	228	228	229	229	230	230	231	231	232	232	233	233	234	234	235	235	236	236	237	237	238	238	239	239	240	240
17	241	241	242	242	243	243	244	244	245	245	246	246	247	247	248	248	249	249	250	250	251	251	252	252	253	253	254	254	255	255
18	256	256	257	257	258	258	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266	267	267	268	268	269	269	270	270
19	271	271	272	272	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280	281	281	282	282	283	283	284	284	285	285
20	P-1b	P-1b	P-2b	P-2b	P-3b	P-3b	Neg	Neg	290	290	291	291	292	292	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300
21	301	301	302	302	303	303	304	304	305	305	306	306	307	307	308	308	309	309	310	310	311	311	312	312	313	313	314	314	315	315
22	316	316	317	317	318	318	319	319	320	320	321	321	322	322	323	323	324	324	325	325	326	326	327	327	328	328	329	329	330	330
23	331	331	332	332	333	333	334	334	335	335	336	336	337	337	338	338	339	339	340	340	341	341	342	342	343	343	344	344	345	345
24	346	346	347	347	348	348	349	349	350	350	351	351	352	352	353	353	354	354	355	355	356	356	357	357	358	358	359	359	360	360
25	361	361	362	362	363	363	364	364	365	365	366	366	367	367	368	368	369	369	370	370	371	371	372	372	373	373	374	374	375	375
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	44	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	509	509	510	510
35	511	511	512	512	513	513	514	514	515	515	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	P-3c	P-3c	P-2c	P-2c	P-1c	P-1c

B. RayBio Human Antibody Array L-507 Target List

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

RayBio Human Antibody Array L-507 Target List… continued

Number	Name	Number	Name	Number	Name	Number	Name
301	IL-22 BP	361	MMP-2	421	RANK / TNFRSF11A	481	TMEFF1 / Tomoregulin-1
302	IL-22 R	362	MMP-3	422	RANTES	482	TMEFF2
303	IL-23	363	MMP-7	423	RELM beta	483	TNF-alpha
304	IL-23 R	364	MMP-8	424	RELT / TNFRSF19L	484	TNF-beta
305	IL-24	365	MMP-9	425	ROBO4	485	TNF RI / TNFRSF1A
306	IL-26	366	MMP-10	426	S100 A8/A9	486	TNF RII / TNFRSF1B
307	IL-27	367	MMP-11 /Stromelysin-3	427	S100A10	487	TRADD
308	IL-28A	368	MMP-12	428	SAA	488	TRAIL / TNFSF10
309	IL-29	369	MMP-13	429	SCF	489	TRAIL R1 / DR4 / TNFRSF10A
310	IL-31	370	MMP-14	430	SCF R /CD117	490	TRAIL R2 / DR5 / TNFRSF10B
311	IL-31 RA	371	MMP-15	431	SDF-1 / CXCL12	491	TRAIL R3 / TNFRSF10C
312	BACE-1	372	MMP-16 / MT3-MMP	432	sFRP-1	492	TRAIL R4 / TNFRSF10D
313	FACX	373	MMP-19	433	sFRP-3	493	TRANCE
314	Insulin	374	MMP-20	434	sFRP-4	494	TREM-1
315	Insulin R	375	MMP-24 / MT5-MMP	435	sgp130	495	TROY / TNFRSF19
316	Insulysin / IDE	376	MMP-25 / MT6-MMP	436	SIGIRR	496	TSG-6
317	IP-10	377	MSP alpha Chain	437	Siglec-5/CD170	497	TSLP R
318	I-TAC / CXCL11	378	Musk	438	Siglec-9	498	TWEAK / TNFSF12
319	Kininostatin / kininogen	379	NAP-2	439	SLPI	499	TWEAK R / TNFRSF12
320	Kremen-1	380	NCAM-1 / CD56	440	Smad 1	500	Ubiquitin+1
321	Kremen-2	381	Neuritin	441	Smad 4	501	uPA
322	Latent TGF-beta bp1	382	NeuroD1	442	Smad 5	502	uPAR
323	LBP	383	Neuropilin-2	443	Smad 7	503	Vasorin
324	Lck	384	Neurturin	444	Smad 8	504	VCAM-1 (CD106)
325	LECT2	385	NGF R	445	Prdx6	505	VE-Cadherin
326	Lefty - A	386	Nidgen-1	446	Soggy-1	506	VEGF
327	Leptin (OB)	387	NOV / CCN3	447	Sonic Hedgehog (Shh N-terminal)	507	VEGF R2 (KDR)
328	Leptin R	388	NrCAM	448	SPARC	508	VEGF R3
329	LFA-1 alpha	389	NRG1 Isoform GGF2	449	Spinesin	509	VEGF-B
330	LIF	390	NRG2	450	TACI / TNFRSF13B	510	VEGF-C
331	LIF R alpha	391	NRG3	451	Tarc	511	VEGF-D
332	LIGHT / TNFSF14	392	NT-3	452	TCCR / WSX-1	512	VEGI / TNFSF15
333	Lipocalin-1	393	NT-4	453	TECK / CCL25	513	WIF-1
334	Lipocalin-2	394	Orexin A	454	TFPI	514	WISP-1 / CCN4
335	LRP-1	395	Orexin B	455	TGF-alpha	515	XEDAR
336	LRP-6	396	OSM	456	TGF-beta 1	516	Neg
337	L-Selectin (CD62L)	397	Osteoactivin / GPNMB	457	TGF-beta 2	517	Neg
338	Lymphotactin / XCL1	398	Osteocrin	458	TGF-beta 3	518	Neg
339	Lymphotoxin beta / TNFSF3	399	Osteoprotegerin / TNFRSF11B	459	TGF-beta 5	519	Neg
340	Lymphotoxin beta R / TNFRSF3	400	OX40 Ligand / TNFSF4	460	TGF-beta RI / ALK-5	520	Neg
341	MAC-1	401	PARC / CCL18	461	TGF-beta RII	521	Neg
342	MCP-1	402	PD-ECGF	462	Grb2	522	Neg
343	MCP-2	403	PDGF R alpha	463	TGF-beta RIII	523	P-3c
344	MCP-3	404	PDGF R beta	464	Thrombopoietin (TPO)	524	P-2c
345	MCP-4 / CCL13	405	PDGF-AA	465	TPX	525	P-1c
346	M-CSF	406	PDGF-AB	466	Thrombospondin-1		
347	M-CSF R	407	PDGF-BB	467	Thrombospondin-2		
348	MDC	408	PDGF-C	468	Thrombospondin-4		
349	MFG-E8	409	PDGF-D	469	Thymopoietin		
350	MFRP	410	PECAM-1 /CD31	470	Tie-1		
351	MICA	411	Pentraxin3 / TSG-14	471	Tie-2		
352	MIF	412	Persephin	472	TIMP-1		
353	MIG	413	PF4 / CXCL4	473	TIMP-2		
354	MIP-1a	414	PlGF	474	TIMP-3		
355	MIP-1b	415	PLUNC	475	TIMP-4		
356	MIP-1d	416	Pref-1	476	TL1A / TNFSF15		
357	MIP 2	417	Progranulin	477	TLR1		
358	MIP-3 alpha	418	Prolactin	478	TLR2		
359	MIP-3 beta	419	P-selectin	479	TLR3		
360	MMP-1	420	RAGE	480	TLR4		

C. RayBio Human Antibody Array L-493 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
2	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26	27	27	28	28	29	29	30	30
3	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41	42	42	43	43	44	44	45	45
4	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60
5	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75
6	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86	87	87	88	88	89	89	90	90
7	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101	102	102	103	103	104	104	105	105
8	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116	117	117	118	118	119	119	120	120
9	121	121	122	122	123	123	124	124	125	125	126	126	127	127	128	128	129	129	130	130	131	131	132	132	133	133	134	134	135	135
10	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146	147	147	148	148	149	149	150	150
11	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160	161	161	162	162	163	163	164	164	165	165
12	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176	177	177	178	178	179	179	180	180
13	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191	192	192	193	193	194	194	195	195
14	196	196	197	197	198	198	199	199	200	200	201	201	202	202	203	203	204	204	205	205	206	206	207	207	208	208	209	209	210	210
15	211	211	212	212	213	213	214	214	215	215	216	216	217	217	218	218	219	219	220	220	221	221	222	222	223	223	224	224	225	225
16	226	226	227	227	228	228	229	229	230	230	231	231	232	232	233	233	234	234	235	235	236	236	237	237	238	238	239	239	240	240
17	241	241	242	242	243	243	244	244	245	245	246	246	247	247	248	248	249	249	250	250	251	251	252	252	253	253	254	254	255	255
18	256	256	257	257	258	258	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266	267	267	268	268	269	269	270	270
19	271	271	272	272	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280	281	281	282	282	283	283	284	284	285	285
20	P-1b	P-1b	P-2b	P-2b	P-3b	P-3b	Neg	Neg	290	290	291	291	292	292	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300
21	301	301	302	302	303	303	304	304	305	305	306	306	307	307	308	308	309	309	310	310	311	311	312	312	313	313	314	314	315	315
22	316	316	317	317	318	318	319	319	320	320	321	321	322	322	323	323	324	324	325	325	326	326	327	327	328	328	329	329	330	330
23	331	331	332	332	333	333	334	334	335	335	336	336	337	337	338	338	339	339	340	340	341	341	342	342	343	343	344	344	345	345
24	346	346	347	347	348	348	349	349	350	350	351	351	352	352	353	353	354	354	355	355	356	356	357	357	358	358	359	359	360	360
25	361	361	362	362	363	363	364	364	365	365	366	366	367	367	368	368	369	369	370	370	371	371	372	372	373	373	374	374	375	375
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	44	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	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	P-3c	P-3c	P-2c	P-2c	P-1c	P-1c

D. RayBio Human Antibody Array L-493 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	Positive-1a	61	ApoE3	121	Ceruloplasmin	181	EpCAM	241	GPR-39
2	Positive-2a	62	ApoD	122	CFHR2	182	EphA1	242	GPX1
3	Positive-3a	63	ApoM	123	Chemerin	183	EphA2	243	GPX3
4	Blank	64	ApoH	124	CHI3L1	184	EphA3	244	Pancreastatin
5	11b-HSD1	65	APP	125	Chromogranin A	185	EphA4	245	GRP
6	2B4	66	ASPH	126	Chymase	186	EphA5	246	GRP75
7	4-1BB	67	Attractin	127	cIAP-2	187	EphA6	247	GRP78
8	ABL1	68	B3GNT1	128	Ck beta 8-1	188	EphA7	248	GSR
9	ACE	69	BAF57	129	CK-MB	189	EphA8	249	GST
10	ACE-2	70	BAFF	130	Claudin-3	190	EphB1	250	HADHA
11	ACK1	71	BAI-1	131	Claudin-4	191	EphB2	251	HAI-1
12	ACPP	72	BCAM	132	CLEC3B	192	EphB3	252	HAI-2
13	ACTH	73	Beta 2M	133	Clusterin	193	EphB4	253	hCG alpha
14	ADAM-9	74	Beta Defensin 4	134	CNDP1	194	EphB6	254	hCGb
15	Neurokinin-A	75	Beta IG-H3	135	Factor XIII A	195	ERRa	255	Hck
16	ADAMTS-1	76	Biglycan	136	Factor XIII B	196	Erythropoietin R	256	HE4
17	ADAMTS-L2	77	BLAME	137	COCO	197	ESAM	257	Hemopexin
18	ADAMTS-4	78	BMP-9	138	C2	198	EV15L	258	Hepcidin
19	ADAMTS-5	79	BMX	139	C3a	199	EXTL2	259	HSP32
20	ADAMTS-10	80	BNIP2	140	C5/C5a	200	FABP1	260	HOXA10
21	ADAMTS-13	81	Btk	141	C7	201	FABP2	261	Haptoglobin
22	ADAMTS-15	82	ApoC1	142	C8B	202	FABP4	262	HSP10
23	ADAMTS-17	83	CA 9	143	C9	203	FAK	263	HSP20
24	ADAMTS-18	84	CA 15-3	144	Complement factor H	204	FAP	264	HSP27
25	ADAMTS-19	85	CA 19-9	145	Contactin-1	205	Fc RIIB/C	265	HSP40
26	Adipsin	86	CA 125	146	Contactin-2	206	Fen 1	266	HSP60
27	Afamin	87	Cadherin-13	147	Corticosteroid-binding globulin	207	FER	267	HSP70
28	AFP	88	Calbindin	148	COX-2	208	Ferritin	268	HSP90
29	ALBUMIN	89	Calbindin D	149	C-peptide	209	Fetuin A	269	HSPA8
30	IL-36RN	90	Calcitonin	150	Creatinine	210	Fetuin B	270	HTRA2
31	Aldolase A	91	Calreticulin	151	CRP	211	FGFR1	271	IBSP
32	Aldolase B	92	Calsyntenin-1	152	CRTAM	212	FGFR1 alpha	272	IGF2BP1
33	Aldolase C	93	CPN2	153	CSH1	213	FGFR2	273	IGFBP-5
34	ALK	94	CART	154	gamma-Thrombin	214	Fibrinogen	274	IL-23p19
35	Alpha Lactalbumin	95	Caspase-3	155	CutA	215	Fibrinopeptide A	275	IL-33
36	Alpha 1 AG	96	Caspase-8	156	cTnT	216	Fibronectin	276	IL-34
37	A1BG	97	Cathepsin B	157	Cyclin D1	217	Ficolin-3	277	INSRR
38	A1M	98	Cathepsin D	158	Cystatin A	218	FIH	278	Integrin alpha V
39	A2M	99	Cathepsin L	159	Cystatin B	219	FOLR1	279	CD61
40	TPM1	100	Cathepsin S	160	Cystatin C	220	FOXN3	280	Itk
41	ALPP	101	CBP	161	Cytochrome C	221	FoxO1	281	ITM2B
42	pro-MMP13	102	CCK	162	Cytokeratin 8	222	FoxP3	282	Kallikrein 2
43	AMICA	103	CD23	163	Cytokeratin 18	223	FRK	283	ApoC3
44	AMPKa1	104	CD24	164	Cytokeratin 19	224	FSH	284	Kallikrein 5
45	Amylin	105	CD36	165	DBI	225	Furin	285	Kallikrein 6
46	ANGPTL3	106	CD38	166	DCBLD2	226	Fyn	286	Positive-1b
47	ANGPTL4	107	CD44	167	D-Dimer	227	GADD45A	287	Positive-2b
48	Annexin A7	108	CD45	168	DEFA1/3	228	Galectin-1	288	Positive-3b
49	APC	109	CD46	169	Defensin	229	Galectin-3BP	289	neg
50	APCS	110	CD47	170	Desmin	230	Galectin-7	290	Kallikrein 7
51	Apelin	111	CD55	171	DLL1	231	Gas1	291	Kallikrein 8
52	Apex1	112	CD59	172	DLL4	232	Gastrin	292	Kallikrein 10
53	APN	113	CD71	173	DMP-1	233	GATA-3	293	Kallikrein 11
54	ApoA1	114	CD74	174	DPPIV	234	GATA-4	294	Kallikrein 14
55	ApoA2	115	CD90	175	BNP	235	Gelsolin	295	KCC3
56	ApoA4	116	CD97	176	E-Cadherin	236	Ghrelin	296	KCTD10
57	ApoB	117	CD 79 alpha	177	Endorphin Beta	237	GLP-1	297	KIF3B
58	ApoC2	118	CD200	178	Endothelin Receptor A	238	GPI	298	KLF4
59	ApoB100	119	CEA	179	Enolase 2	239	GPBB	299	LAG-3
60	ApoE	120	CEACAM-1	180	ENPP2	240	GMNN	300	pro-Glucagon

RayBio® Human Antibody Array L-493 Target List ...continued

Number	Name	Number	Name	Number	Name	Number	Name
301	Layilin	361	Pappalysin-1	421	S100A4	481	TRPC6
302	LDL R	362	Pancreatic Polypeptide	422	S100A6	482	TRPM7
303	Legumain	363	Presenilin 1	423	S100A8	483	Trypsin 1
304	LH	364	PARK7	424	S-100b	484	TSH
305	LIMPII	365	Visfatin	425	SART1	485	TSLP
306	LIN41	366	P-Cadherin	426	SART3	486	TXK
307	Livin	367	PCAF	427	SCG3	487	Uromodulin
308	LOX-1	368	PD-1	428	Selenoprotein P	488	TFF1
309	LPS	369	PTH	429	SEMA3A	489	VDUP-1
310	LRG1	370	Troponin C	430	Serotonin	490	VEGF R1
311	LTF	371	PDX-1	431	Serpin A1	491	VGF
312	LTK	372	PEDF	432	Serpin A12	492	VIP Receptor 2
313	Lumican	373	PEPSINOGEN I	433	Serpin A3	493	Vitamin D Receptor
314	Lyn	374	PEPSINOGEN II	434	Serpin A4	494	Vitamin D-BP
315	LYRIC	375	Vasopressin	435	Serpin A5	495	Vitamin K-dependent protein S
316	LYVE-1	376	PGRP-S	436	Serpin A8	496	Vitronectin
317	LZTS1	377	PI 16	437	Serpin A9	497	VWF
318	Mammaglobin A	378	PI 3Kinase p85 beta	438	Serpin B5	498	Wilms Tumor 1
319	Marapsin	379	PIM2	439	Serpin D1	499	XIAP
320	MATK	380	PKM2	440	Serpin I1	500	ZAG
321	MBL	381	Plasminogen	441	SERPING1	501	ZAP70
322	MBL-2	382	Podocalyxin	442	SERTAD2	502	Neg
323	Mer	383	POMC	443	SHBG	503	Neg
324	Mesothelin	384	PON1	444	SMAC	504	Neg
325	MICB	385	PON2	445	SNCG	505	Neg
326	Midkine	386	PPARg2	446	SSTR5	506	Neg
327	MINA	387	PPP2R5C	447	Somatotropin	507	Neg
328	FABP3	388	NR3C3	448	SOST	508	Positive-3c
329	MSHa	389	INSL3	449	SOX17	509	Positive-2c
330	MTUS1	390	Pro-BDNF	450	SOX2	510	Positive-1c
331	Myoglobin	391	Procalcitonin	451	SPARCL1		
332	NAIP	392	Pro-Cathepsin B	452	SPINK1		
333	Nanog	393	Thrombin	453	SRMS		
334	NELL2	394	Prohibitin	454	SSEA-1		
335	NEP	395	ProSAAS	455	SSEA-4		
336	Galanin	396	Prostasin	456	SSTR2		
337	Nesfatin	397	PSP	457	Survivin		
338	Nestin	398	Pro-MMP-7	458	SYK		
339	NET1	399	Pro-MMP-9	459	Syndecan-1		
340	Netrin G2	400	Protein p65	460	Syndecan-3		
341	Netrin-4	401	PSA-Free	461	TACE		
342	Neuropeptide Y	402	PSA-total	462	TAF4		
343	NF1	403	PTHLP	463	Tyk2		
344	NM23-H1/H2	404	PTN	464	Tec		
345	Presenilin 2	405	PTPRD	465	TFF3		
346	Notch-1	406	PYK2	466	Thrombomodulin		
347	NPTX1	407	PYY	467	Thymidine Kinase-1		
348	NPTXR	408	Ras	468	Thyroglobulin		
349	Progesterone	409	RBP4	469	TIM-1		
350	Ntn1	410	RECK	470	TNK1		
351	OCT3/4	411	RELM alpha	471	TOPORS		
352	Omentin	412	Resistin	472	TPA		
353	Osteocalcin	413	RET	473	TRA-1-60		
354	Osteopontin	414	RIP1	474	TRA-1-81		
355	OX40	415	ROCK1	475	Transferrin		
356	p21	416	ROCK2	476	Trappin-2		
357	p27	417	ROR1	477	TRKB		
358	p53	418	ROR2	478	TROPONIN I		
359	PAI-1	419	ROS	479	TYRO10		
360	PAK7	420	RYK	480	TRPC1		

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.

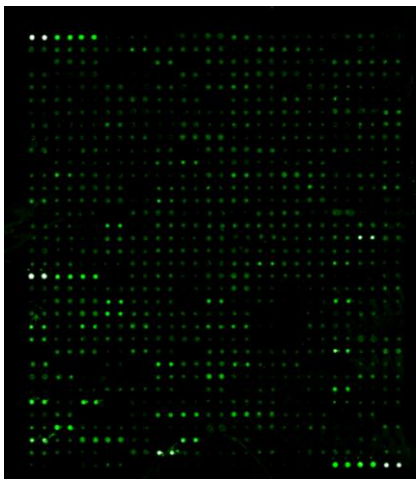
2) Negative Control (NEG) spots contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical Results

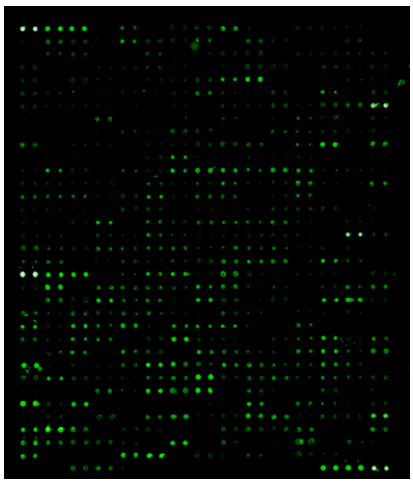
The following figure shows the RayBio® L-Series Human Antibody Array 1000 probed with a serum sample. The images were captured using a Axon GenePix laser scanner. The strong signals in row 20 and the upper left and lower right corners of each array are Positive Controls, which can be used to identify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

RayBio® L-series Human Antibody Array 507

Sample-1

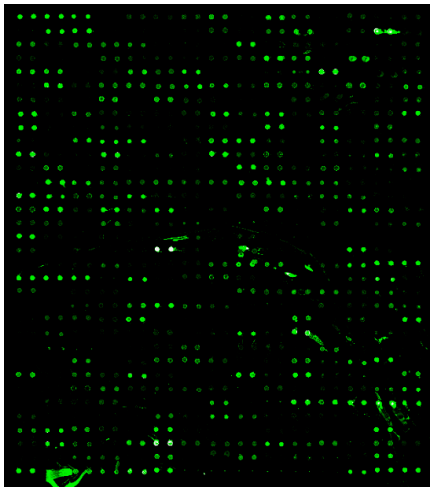


Sample-2

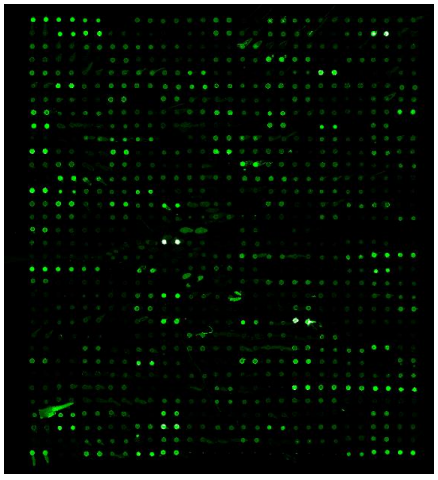


RayBio® L-series Human Antibody Array L-493

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

Christina Scheel et all., *Paracrine and Autocrine Signals Induce and Maintain Mesenchymal and Stem Cell States in the Breast*. Cell. 2011;145, 926–940

Lin Y, Huang R, Chen L, et al., *Profiling of cytokine expression by biotin-labeled-based protein arrays*. Proteomics. 2003, 3: 1750–1757.

Huang R, Jiang W, Yang J, et al., *A Biotin Label-based Antibody Array for High-content Profiling of Protein Expression*. Cancer Genomics Proteomics. 2010; 7(3):129–141.

Liu T, Xue R, Dong L, et al., *Rapid determination of serological cytokine biomarkers for hepatitis B–virus-related hepatocellulare carcinoma using antibody arrays*. Acta Biochim Biophys Sin. 2011; 43(1):45–51.

Cui J, Chen Y, Chou W-C, et al., *An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer*. Nucl Acids Res. 2011; 39(4):1197–1207.

Jun Zhong et all., *Temporal Profiling of the Secretome during Adipogenesis in Humans*. Journal of Proteome Research. 2010, 9, 5228–5238

Chowdury UR, Madden BJ, Charlesworth MC, Fautsch MP., *Proteomic Analysis of Human Aqueous Humor*. Invest Ophthalmol Visual Sci. 2010; 51(10):4921–4931.

Wei Y, Cui C, Lainscak M, et al., *Type-specific dysregulation of matrix metalloproteinases and their tissue inhibitors in end-stage heart failure patients: relationshp between MMP-10 andLV remodeling*. J Cell Mol Med. 2011; 15(4):773–782.

Kuranda K, Berthon C, Lepêtre F, et al., *Expression of CD34 in hematopoietic cancer cell lines reflects tightly regulated stem/progenitor-like state*. J Cell Biochem. 2011; 112(5):1277–1285.

Toh HC, Wang W-W, Chia WK, et al., *Clinical Benefit of Allogenic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients*. Clin Chem Res. 2009; 15:7726–7736.

Zhen Hou, *Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosi*. Biomarkers. 2009;14(8): 604-618.

Yao Liang Tang, et al., *Hypoxic Preconditioning Enhances the Benefit of Cardiac Progenitor Cell Therapy for Treatment of Myocardial Infarction by Inducing CXCR4*. Circ Res. 2009;109:197723

RayBio® L-series Antibody Arrays are patent-pending technology developed by RayBiotech.

This product is intended for research only and is not to be used for clinical diagnosis. Our produces may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for six months from the date of shipment when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

RayBio® is a registered trademark of RayBiotech, Inc.

GenePix® is a registered trademark of Molecular Devices, Inc.

This product is for research use only.