RayBio® Label-Based (L-Series) Mouse Antibody Array L-1308 Glass Slide Kit

A combination of Mouse L-308, L-2, and L-3 arrays

Patent Pending Technology
User Manual (January 1, 2022)

For the simultaneous detection of the relative expression of 1308 mouse proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

L-Series Mouse Antibody Array L-1308
Cat# AAM-BLG-1308-4 (4 Sample Kit)
Cat# AAM-BLG-1308-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

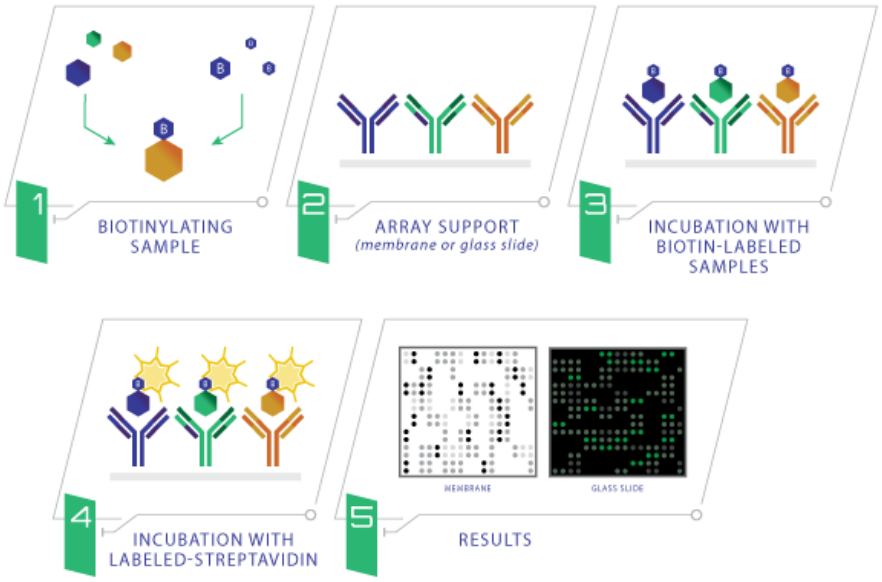
Tel: (Toll Free) 1-888-494-8555 or +1-770-729-2992; Fax: +1-770-206-2393; Website: www.raybiotech.com Email: info@raybiotech.com

TABLE OF CONTENTS

I. Introduction

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

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



II. Materials Provided

A. Storage Recommendations

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

ITEM	DESCRIPTION	4 SAMPLE Kit	8 SAMPLE Kit
A	Spin Columns (0.5ml)	16 columns	32 columns
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	2 vials (50 µl)
E	RayBio® L-Series Glass Slide*	1 slide each of Mouse L-308, L-2, and L-3	2 slides each of Mouse L-308, L-2, and L-3
F	Blocking Buffer	1 bottle (30 ml)	2 bottles (30 ml)
G	20X Wash Buffer I	2 bottles (30 ml)	3 bottles (30 ml)
H	20X Wash Buffer II	2 bottles (30 ml)	3 bottles (30 ml)
I	Cy3 equivalent-Conjugated Streptavidin	2 vials	3 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (30 ml)	2 bottles (30 ml)
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	1 bottle (10 ml)
M	30 ml Centrifuge Tube	1 tube	2 tubes

*Each slide contains 4 identical subarrays

**Only needed if testing cell or tissue lysates

B. Additional Materials Required

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

III. Overview and General Considerations

A. Preparation and Storage of Samples

1) Preparation of Cell Culture Supernatants
   1. Seed cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes.*
   2. Culture cells in complete culture medium for ~24–48 hours.**
   3. Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours.**,† The membrane-based array is recommended if high serum medium such as 10% FCS/FBS is used, as high background can occur on glass slide arrays with high serum containing media samples.
   4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as ≤1 ml aliquots at -80°C until needed.
   5. If you want to use cell mass for inter-sample normalization, measure the total wet weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can normalize between arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

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

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

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

2) Extracting Protein from Cells

1. Centrifuging Cells

    a. Adherent Cells:

        i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.

        ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.

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

2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.

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

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

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

3) Extracting Protein from Crude Tissue

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

2. Homogenize the tissue according to homogenizer manufacturer instructions.

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

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

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

4) Determine the total protein concentration

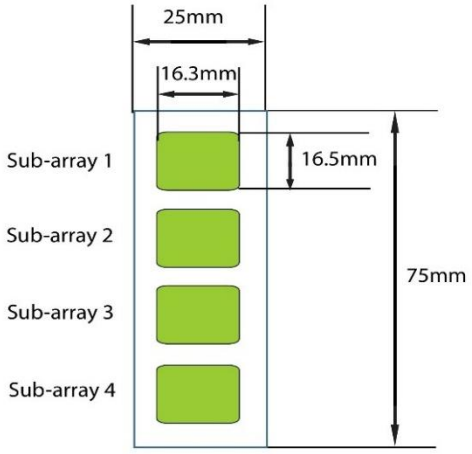
For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

B. Handling the Glass Slides

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

C. Layout of Array Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

D. Incubations and Washes

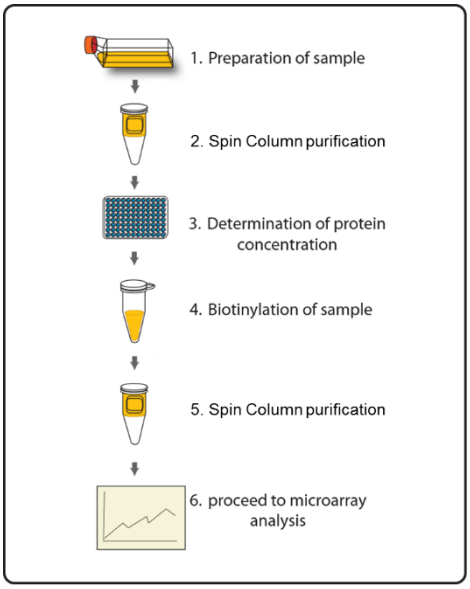
- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.

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

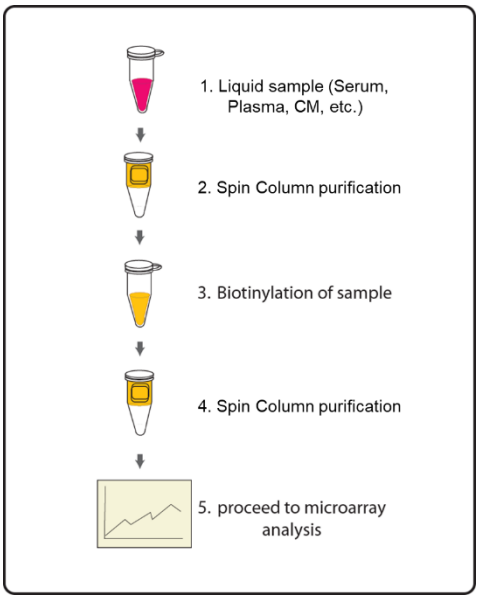
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



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



A. Sample purification

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

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).

2. Place the Spin column into a collection tube, centrifuge at 1,500 x g for 1 minute to remove the storage buffer. Discard the flow-through.

3. Wash the Spin Column three times with 300 µl Labeling Buffer each, centrifuge at 1,500 x g for 1 minute to remove the flow-through. Discard the flow-through and blot the bottom of the column to

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

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

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

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

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

B. Biotin-Labeling the Sample

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

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

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

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

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

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

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

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

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

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

C. Drying the Glass Slide

8. Remove the package containing the Assembled Glass Slide (Item E) from the freezer. Place unopened package on the bench top for ~15 minutes, and allow the Assembled Glass Slide to equilibrate to RT.

9. Open package, and take the Assembled Glass Slide out of the sleeve. Do not disassemble the Glass Slide from the chamber assembly. Place glass slide assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubations

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

10. Block sub-arrays by adding 400 µl of Blocking Buffer (Item F) into each well of Assembled Glass Slide and incubating at RT for 30 minutes. Ensure there are no bubbles on the array surfaces.

11. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 10-fold for cell culture supernatants, 20-fold for serum/plasma and 100-fold for cell/tissue lysate. *Dilution for other body fluid needs to be determined by the end user. Generally, most samples can be 10-20x dilution, while tears and saliva samples may need 100x dilution.*

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

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

Note: Avoid the flow of sample into neighboring wells.

13. Based on number of samples and remaining protocol, calculate the amount of 1X Wash Buffer I and 1X Wash Buffer II needed to complete the experiment. Separately dilute the required amounts of 20X Wash Buffer I Concentrate (Item G) 20-fold and 20X Wash Buffer II Concentrate (Item H) with ddH_2O.

14. Decant the samples from each well and wash 3 times with 800 µl of 1X Wash Buffer I at RT with gentle rocking or shaking for 5 minutes per wash.

15. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer I to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 10 minutes per wash.

16. Decant the Wash Buffer I from each well, place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer II to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 5 minutes per wash.

17. Prepare 1X Cy3-Conjugated Streptavidin:

    a) Briefly spin down tube containing the Cy3-Conjugated Streptavidin (Item I) immediately before use.

    b) Add 1000 µl of Blocking Buffer into the Cy3-Conjugated Streptavidin tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).

    c) To prepare 1X Cy3-Conjugated Streptavidin, add 200 µl of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800 µl of Blocking Buffer. Mix gently.

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

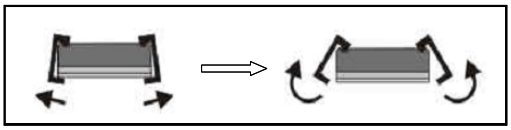
Note: Avoid exposure to light in Steps 19–25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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

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

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

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



21. Gently place the glass slide into 30 ml Centrifuge Tube (Item M). Add enough 1X Wash Buffer I to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 10 min. Remove the wash buffer. Repeat 2 times for a total of 3 washes.

22. Add enough 1X Wash Buffer II to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 5 minutes. Remove the wash buffer. Repeat one time for a total of two washes for 5 minutes per wash.

23. Finally, wash the glass slide with 30 ml of ddH_2O for 5 minutes. Remove glass slide and decant water from Centrifuge Tube.

24. Remove buffer droplets from the slide completely by one of the following ways:

    - Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
    - Or dry the glass slide by a compressed N2 stream.
    - Or gently apply suction with a pipette to remove buffer droplets. Do not touch the array surface, only the sides.

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

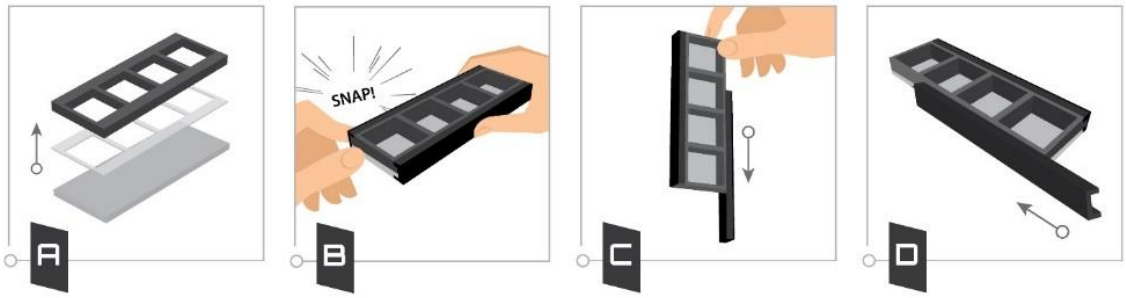
E. Fluorescence Detection

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

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

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

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



V. Antibody Array Map

A. RayBio® Mouse Antibody Array L-308 Target List

	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
1	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10
2	11	11	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24
3	25	25	26	26	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38
4	39	39	40	40	41	41	42	42	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52
5	53	53	54	54	55	55	56	56	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66
6	67	67	68	68	69	69	70	70	71	71	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80
7	81	81	82	82	83	83	84	84	85	85	86	86	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94
8	95	95	96	96	97	97	98	98	99	99	100	100	101	101	102	102	103	103	104	104	105	105	106	106	107	107	108	108
9	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116	117	117	118	118	119	119	120	120	121	121	122	122
10	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	136	136
11	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
12	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160
13	161	161	162	162	163	163	164	164	165	165	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174
14	175	175	176	176	177	177	178	178	179	179	180	180	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188
15	189	189	190	190	191	191	192	192	193	193	194	194	195	195	196	196	197	197	198	198	199	199	200	200	201	201	202	202
16	203	203	204	204	205	205	206	206	207	207	208	208	209	209	210	210	211	211	212	212	213	213	214	214	215	215	216	216
17	217	217	218	218	219	219	220	220	221	221	222	222	223	223	224	224	225	225	226	226	227	227	228	228	229	229	230	230
18	231	231	232	232	233	233	234	234	235	235	236	236	237	237	238	238	239	239	240	240	241	241	242	242	243	243	244	244
19	245	245	246	246	247	247	248	248	249	249	250	250	251	251	252	252	253	253	254	254	255	255	256	256	257	257	258	258
20	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266	267	267	268	268	269	269	270	270	271	271	272	272
21	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	286	286
22	287	287	288	288	289	289	290	290	291	291	292	292	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300
23	301	301	302	302	303	303	304	304	305	305	306	306	307	307	308	308	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

B. RayBio® Mouse Antibody Array L-2 and L-3 Target List

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11
2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
3	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41
4	42	42	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56
5	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71
6	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86
7	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101
8	102	102	103	103	104	104	105	105	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116
9	117	117	118	118	119	119	120	120	121	121	122	122	123	123	124	124	125	125	126	126	127	127	128	128	129	129	130	130	131	131
10	132	132	133	133	134	134	135	135	136	136	137	137	138	138	139	139	140	140	141	141	142	142	143	143	144	144	145	145	146	146
11	147	147	148	148	149	149	150	150	151	151	152	152	153	153	154	154	155	155	156	156	157	157	158	158	159	159	160	160	161	161
12	162	162	163	163	164	164	165	165	166	166	167	167	168	168	169	169	170	170	171	171	172	172	173	173	174	174	175	175	176	176
13	177	177	178	178	179	179	180	180	181	181	182	182	183	183	184	184	185	185	186	186	187	187	188	188	189	189	190	190	191	191
14	192	192	193	193	194	194	195	195	196	196	197	197	198	198	199	199	200	200	201	201	202	202	203	203	204	204	205	205	206	206
15	207	207	208	208	209	209	210	210	211	211	212	212	213	213	214	214	215	215	216	216	217	217	218	218	219	219	220	220	221	221
16	222	222	223	223	224	224	225	225	226	226	227	227	228	228	229	229	230	230	231	231	232	232	233	233	234	234	235	235	236	236
17	237	237	238	238	239	239	240	240	241	241	242	242	243	243	244	244	245	245	246	246	247	247	248	248	249	249	250	250	251	251
18	252	252	253	253	254	254	255	255	256	256	257	257	258	258	259	259	260	260	261	261	262	262	263	263	264	264	265	265	266	266
19	267	267	268	268	269	269	270	270	271	271	272	272	273	273	274	274	275	275	276	276	277	277	278	278	279	279	280	280	281	281
20	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	282	282	283	283	284	284	285	285	286	286	287	287	288	288	289	289	290	290	291	291	292	292
21	293	293	294	294	295	295	296	296	297	297	298	298	299	299	300	300	301	301	302	302	303	303	304	304	305	305	306	306	307	307
22	308	308	309	309	310	310	311	311	312	312	313	313	314	314	315	315	316	316	317	317	318	318	319	319	320	320	321	321	322	322
23	323	323	324	324	325	325	326	326	327	327	328	328	329	329	330	330	331	331	332	332	333	333	334	334	335	335	336	336	337	337
24	338	338	339	339	340	340	341	341	342	342	343	343	344	344	345	345	346	346	347	347	348	348	349	349	350	350	351	351	352	352
25	353	353	354	354	355	355	356	356	357	357	358	358	359	359	360	360	361	361	362	362	363	363	364	364	365	365	366	366	367	367
26	368	368	369	369	370	370	371	371	372	372	373	373	374	374	375	375	376	376	377	377	378	378	379	379	380	380	381	381	382	382
27	383	383	384	384	385	385	386	386	387	387	388	388	389	389	390	390	391	391	392	392	393	393	394	394	395	395	396	396	397	397
28	398	398	399	399	400	400	401	401	402	402	403	403	404	404	405	405	406	406	407	407	408	408	409	409	410	410	411	411	412	412
29	413	413	414	414	415	415	416	416	417	417	418	418	419	419	420	420	421	421	422	422	423	423	424	424	425	425	426	426	427	427
30	428	428	429	429	430	430	431	431	432	432	433	433	434	434	435	435	436	436	437	437	438	438	439	439	440	440	441	441	442	442
31	443	443	444	444	445	445	446	446	447	447	448	448	449	449	450	450	451	451	452	452	453	453	454	454	455	455	456	456	457	457
32	458	458	459	459	460	460	461	461	462	462	463	463	464	464	465	465	466	466	467	467	468	468	469	469	470	470	471	471	472	472
33	473	473	474	474	475	475	476	476	477	477	478	478	479	479	480	480	481	481	482	482	483	483	484	484	485	485	486	486	487	487
34	488	488	489	489	490	490	491	491	492	492	493	493	494	494	495	495	496	496	497	497	498	498	499	499	500	500	Neg	Neg	Neg	Neg
35	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

VI. Antibody Array Target List

A. RayBio® Mouse Antibody Array L-308 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	6Ckine	63	DPPIV	125	IGFBP-1	187	IL-28B	249	SCF R
2	Activin A	64	DR3	126	IGFBP-2	188	IL-31	250	SDF-1
3	Activin C	65	Dtk	127	IGFBP-3	189	IL-31 RA	251	SAA1
4	Activin R1B	66	EDAR	128	IGFBP-5	190	Insulin	252	Shh-N
5	Adiponectin	67	EGFR	129	IGFBP-6	191	Integrin beta-2	253	SIGIRR
6	AgRP	68	EG-VEGF	130	IGFBP-L1	192	I-TAC	254	SLPI
7	ALCAM	69	Endocan	131	IGF-1	193	GRO alpha	255	Soggy-1
8	ANGPTL2	70	Endoglin	132	IGF-2	194	Kremen-1	256	SPARC
9	ANGPTL3	71	Endostatin	133	IL-1 alpha	195	Kremen-2	257	Spinesin
10	Amphiregulin	72	Eotaxin-1	134	IL-1 beta	196	Lefty-1	258	TACI
11	Artemin	73	Eotaxin-2	135	IL-1 R4	197	Leptin R	259	TARC
12	Axl	74	Epigen	136	IL-1 R6	198	LEPTIN	260	TCA-3
13	bFGF	75	Epiregulin	137	IL-1 R9	199	LIF	261	IL-27 R alpha
14	B7-1	76	Erythropoietin	138	IL-1 R1	200	LIGHT	262	TECK
15	BAFF R	77	E-Selectin	139	IL-1 R2	201	LIX	263	TFPI
16	BCMA	78	FADD	140	IL-2	202	LRP-6	264	TGF beta 1
17	beta-Catenin	79	FAM3B	141	IL-2 R alpha	203	L-Selectin	265	TGF beta 2
18	BLC	80	Fas	142	IL-2 R beta	204	Lungkine	266	TGF beta 3
19	Betacellulin	81	Fas Ligand	143	IL-3	205	Lymphotactin	267	TGF beta R1
20	Cardiotrophin-1	82	Fc gamma RIIB	144	IL-3 R alpha	206	LTBR	268	TGF beta R2
21	IL-1ra	83	FGF R3	145	IL-3 R beta	207	MAdCAM-1	269	TSP-1
22	CCL28	84	FGF R4	146	IL-4	208	MCP-1	270	CXCL7
23	MIP-1 beta	85	FGF R5 beta	147	IL-4 R	209	MCP-5	271	Tie-2
24	MCP-3	86	FGF-21	148	IL-5	210	M-CSF	272	TIMP-1
25	MCP-2	87	Flt-3 Ligand	149	IL-5 R alpha	211	MDC	273	TIMP-2
26	CCR10	88	FLRG	150	IL-6	212	MFG-E8	274	TIMP-4
27	CCR3	89	Follistatin-like 1	151	IL-6 R	213	MFRP	275	TL1A
28	CCR4	90	Fractalkine	152	IL-7	214	MIG	276	TLR1
29	CCR6	91	Frizzled-1	153	IL-7 R alpha	215	MIP-1 alpha	277	TLR2
30	CCR7	92	Frizzled-6	154	IL-9	216	MIP-1 gamma	278	TLR3
31	CCR9	93	Frizzled-7	155	IL-9 R	217	MIP-2	279	TLR4
32	CD11b	94	Galectin-3	156	IL-10	218	MIP-3 alpha	280	TMEFF1
33	CD14	95	GCSF	157	IL-10 R alpha	219	MIP-3 beta	281	TNF RI
34	CRP	96	GDF-1	158	IL-11	220	MMP-2	282	TNF RII
35	CD27	97	GDF-3	159	IL-12 p40	221	MMP-3	283	TNF alpha
36	CD27 Ligand	98	GDF-5	160	IL-12 p70	222	MMP-9	284	TNF beta
37	CD30	99	GDF-8	161	IL-12 R beta 1	223	MMP-12	285	Thrombopoietin
38	CD30 Ligand	100	GDF-9	162	IL-13	224	MMP-14	286	TRAIL
39	CD40	101	GFR alpha-2	163	IL-13 R alpha 2	225	MMP-24	287	TRAIL R2
40	CD40 Ligand	102	GFR alpha-3	164	IL-15	226	NRG3	288	TRANCE
41	Cerberus 1	103	GFR alpha-4	165	IL-15 R alpha	227	Neurturin	289	TREM-1
42	Chordin-Like 2	104	GITR	166	IL-16	228	NGFR	290	TROY
43	F3	105	GITR Ligand	167	IL-17A	229	NOV	291	TSLP
44	IL-2 R gamma	106	Glut2	168	IL-17 RB	230	Osteoactivin	292	TSLP R
45	IP-10	107	GM-CSF	169	IL-17C	231	Osteopontin	293	TWEAK
46	Cripto-1	108	Granzyme B	170	IL-17D	232	Osteoprotegerin	294	TWEAK R
47	Crossveinless-2	109	Granzyme D	171	IL-17E	233	OX40 Ligand	295	Ubiquitin+1
48	Cryptic	110	Granzyme G	172	IL-17F	234	PDGF-C	296	uPAR
49	CSK	111	Gremlin-1	173	IL-17 RA	235	PDGF R alpha	297	Urokinase
50	CTACK	112	GHR	174	IL-17 RC	236	PDGF R beta	298	VCAM-1
51	CTLA-4	113	HGFR	175	IL-17 RD	237	Pentraxin-3	299	VE-Cadherin
52	CXCL14	114	HGF	176	IL-18 R alpha	238	PF4	300	VEGF-A
53	CXCL16	115	HVEM	177	IL-20	239	PlGF-2	301	VEGFR1
54	CXCR2	116	ICAM-1	178	IL-20 R alpha	240	Progranulin	302	VEGFR2
55	CXCR3	117	ICAM-2	179	IL-21	241	Prolactin	303	VEGFR3
56	CXCR4	118	ICAM-5	180	IL-21 R	242	P-Selectin	304	VEGF-B
57	CXCR6	119	ICK	181	IL-22	243	RAGE	305	VEGF-C
58	EGF	120	IFN-alpha/beta R1	182	IL-22BP	244	RANTES	306	VEGF-D
59	Decorin	121	IFN-alpha/beta R2	183	IL-23	245	RELM beta	307	WIF-1
60	DKK-1	122	IFN-beta	184	IL-23 R	246	Resistin	308	WISP-1
61	Dkk-3	123	IFN-gamma	185	IL-24	247	S100A10		
62	Dkk-4	124	IFN-gamma R1	186	IL-27	248	SCF		

B. RayBio® Mouse Antibody Array L-2 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 beta	73	ASGR2	145	CD21	217	D4	289	Fodrin alpha	361	hnRNP A2B1	433	Lubricin
2	14-3-3 zeta	74	ASH2L	146	CD39L4	218	DAN	290	Frizzled 8	362	hnRNP C1+C2	434	LUZP1
3	53BP1	75	ASL	147	CD41	219	DARS2	291	FRY	363	hnRNP G	435	LYZL1
4	AMY1	76	AspAT	148	CD42b	220	DBH	292	FSH-B	364	hnRNP L	436	MAGI2
5	AAT1	77	DNPEP	149	CD48	221	DCXR	293	FTL1	365	hnRNP M	437	MAN1
6	ABAT	78	ASXL1	150	CD5L	222	DDAH1	294	FUCA2	366	hnRNP U	438	MAN1A1
7	ABCF1	79	ATP5A1	151	CD98	223	DDT	295	FUS	367	Hornerin	439	Mannosidase II
8	ABI3BP	80	ATPB	152	CDA	224	DDX3Y	296	G3BP1	368	Hoxb3	440	MAP1A
9	ACAA1	81	B3GNT2	153	CDK2	225	DEFA6	297	G6PD	369	HOXD11	441	MAPRE1
10	ACAA2	82	B4GalT1	154	CED-6	226	Desmocollin 1	298	GALNT2	370	HP1BP3	442	MARCKS
11	ACACA	83	B7-H2	155	CENPF	227	Desmocollin-2	299	GANAB	371	HPD	443	MASP3
12	ACLY	84	BAD	156	CEP57	228	Desmocollin-3	300	GAPDH	372	HPRT1	444	MBD2
13	ACO1	85	BASP1	157	CES1	229	Desmoglein-1	301	GARNL1	373	HRG	445	MBP
14	ACTBL2	86	Bassoon	158	Cezanne	230	Desmoglein-2	302	GART	374	HRP12	446	MCAM
15	ACTC1	87	Bcl2l2	159	CFB	231	Desmoplakin 3	303	Gastrokine 1	375	HSPA1A	447	Mcl-1
16	ACTG1	88	BCoR	160	CFHR1	232	DGK-theta	304	GATM	376	HTRA1	448	MCM
17	ACTG2	89	beta I Spectrin	161	CFI	233	DISC 1	305	GBE1	377	HUWE1	449	MDH1
18	ACTN1	90	beta I Tubulin	162	CFVII	234	DMRN9	306	GCDFP 15	378	IDH1	450	MEP1A
19	ADA	91	beta III Tubulin	163	Chitobiase	235	DOT1L	307	GCLC	379	IFRD1	451	MT-2
20	ADAMDEC1	92	BID	164	Chitotriosidase	236	DPP3	308	GCSH	380	IGF2BP2	452	Metavinculin
21	ADAS	93	BIN2	165	Cholinesterase	237	DRIL1	309	GDA	381	IGFBP7	453	MFAP4
22	ADGRF5	94	Biotinidase	166	CHORDC1	238	DSCAM	310	GDF7	382	IGSF4B	454	MFI2
23	ADGRL4	95	BIRC6	167	CHREBP	239	DSPG3	311	GDI1	383	ILK	455	mGLUR5
24	ADH1	96	BMP-1	168	Chromogranin B	240	ECHS1	312	GDI2	384	Inhibin beta	456	Mimecan
25	ADH1C	97	BPGM	169	CKB	241	ECI1	313	Gephyrin	385	Integrin b1	457	MLCK
26	ADH4	98	BPIFB1	170	CLIC1	242	ECM1	314	GFAP	386	Integrin beta 6	458	MMR
27	ADH5	99	BPIFB2	171	CLIP1	243	EEF1G	315	GGCT	387	Intergrin a6	459	MN1
28	ADM	100	Brevican	172	CL-P1	244	EEF2	316	GGH	388	IQGAP2	460	Moesin
29	Advillin	101	BRG1	173	CLTA	245	EFEMP2	317	GIP	389	IRE1	461	MP1
30	AEBP1	102	BRSK1	174	CNOT1	246	EFTUD2	318	GLIPR2	390	IRS2	462	MPCA
31	AFG3L2	103	C1QA	175	CO4A2	247	EHD3	319	GLUD1	391	ISOC2	463	MPO
32	AGA	104	C1QB	176	Cofilin-1	248	Eif4a1	320	Glycoprotein V	392	ITGB4BP	464	MRP 1
33	Aggrecan	105	C1QR	177	COG4	249	ELAVL1	321	GM2A	393	ITIH2	465	MSH6
34	Agrin	106	C1RL	178	COL19A1	250	EMSY	322	GMF beta	394	ITIH3	466	Mtor
35	AGXT	107	C1s	179	COL4A3	251	EN2	323	GNB1	395	ITIHC4	467	Multimerin 2
36	Ahsp	108	C4BPA	180	Col6A2	252	Endorepellin	324	GNPTG	396	JAM-A	468	MyBPC3
37	AIFM1	109	C6	181	COL9A3	253	ENO3	325	GOLIM4	397	JPT1	469	MYH2
38	AKAP9	110	C8A	182	COLEC10	254	ENSA	326	GOLM1	398	KDM4B	470	MYH6
39	AKR1B1	111	C8G	183	Collagen I a1	255	EPB41	327	GPD1	399	Keratin 36	471	MYH7
40	AKR7A2	112	C9orf40	184	Collagen III	256	EPCR	328	GPLD1	400	KIAA0319L	472	MYHC 2x
41	ALAD	113	CA1	185	Collagen IVa6	257	Ephrin B1	329	GRHPR	401	KIAA1468	473	MYL12B
42	ALDH16A1	114	CA150	186	Collagen IX	258	Eps 15	330	GRP170	402	KLKB1	474	MYO5A
43	ALDH1A1	115	CACNB4	187	Collagen V	259	ERAB	331	GSS	403	KMT2D	475	Myoferlin
44	ALDH9A1	116	Cadherin 22	188	Collagen X	260	ERp29	332	GSTM1	404	KRT31	476	Myosin 18B
45	alpha Actinin 4	117	Cadherin-6	189	Collagen XV	261	ERp57	333	GSTO1	405	KRT33B	477	Myosin9
46	alpha Synuclein	118	CALD1	190	COMP	262	ERp72	334	GSTP1	406	KRT73	478	NABC1
47	alpha Tubulin 4	119	Calpain S1	191	Corneodesmosin	263	ESD	335	Guanylin	407	KRT82	479	NAGLU
48	ALPL	120	Calpastatin	192	Cortactin	264	ESR1	336	GZMM	408	KRT85	480	NAP1L1
49	ALS	121	Calponin-2	193	COTL1	265	Ezrin	337	H6PD	409	KSR1	481	NAPRT1
50	Alsin	122	Calretinin	194	CPB2	266	FABP5	338	HABP2	410	LAF4	482	NASP
51	Aminoacylase 1	123	Calumenin	195	CPE	267	Factor IX	339	HBB	411	LAIR1	483	NCAM2
52	Aminopeptidase A	124	CAP1	196	CPEB3	268	Factor V	340	HDGF	412	LAMB1	484	Nebulin
53	Androgen Receptor	125	CAPZA1	197	CPM	269	Factor XI	341	Hemoglobin	413	LMNA	485	Nectin-1
54	ANGPTL6	126	CA2	198	CPNE3	270	Factor XII	342	Hemoglobin A1c	414	LMNB2	486	Nectin-3
55	ANGPTL8	127	CA3	199	CRHBP	271	Factor XIII	343	HEXB	415	LAMA2	487	Neogenin
56	Ankrd26	128	Caspase-14	200	CrkL(1)	272	FAH	344	HGFA	416	LAMB2	488	Nesprin2
57	Annexin A1	129	Catalase	201	CRMP2	273	FAM20C	345	HIBADH	417	LAMC1	489	Neurofibromin
58	Annexin A2	130	Cathelicidin	202	CRTAC1	274	FAM3C	346	HINT1	418	LAMP1	490	Neurogranin
59	Annexin A5	131	Cathepsin A	203	CRYZ	275	FASN	347	HIP1R	419	LASP1	491	Neuropeptide B
60	Annexin A6	132	Cathepsin G	204	Cyclophilin A	276	FASTKD5	348	Histone H1.2	420	LCAT	492	Neuropilin-1
61	ANP	133	Cathepsin H	205	Cyclophilin B	277	FBP 38	349	Histone H1.4	421	LCMT2	493	Neurotrimin
62	ANP32A	134	Cathepsin Z	206	Cystatin	278	FDPS	350	Histone H2A	422	LDH-H	494	NF-M
63	Antithrombin III	135	CBS	207	CYTL1	279	FGG	351	Histone H2A.Z	423	LEDGF	495	NIF3L1
64	APLP1	136	CCAR2	208	Cytochrome b5	280	Fibrillin 1	352	Histone H2B K	424	Limbin	496	NME3
65	AQR	137	CCDC126	209	Cytochrome c	281	Fibrinogen-like 2	353	Histone H3.3	425	LIMS1	497	nNOS1
66	ARFGEF3	138	CCDC25	210	Cytokeratin 1	282	Fibrinopeptide B	354	Histone H4	426	LMW-PTP	498	Notch-2
67	Arp3	139	CCS	211	Cytokeratin 10	283	Fibulin 3	355	HMGB1	427	LOK	499	NPAS3
68	ARPC2	140	CD109	212	Cytokeratin 13	284	Ficolin 2	356	HMGB2	428	LOX	500	NPM1
69	ARPC3	141	CD133	213	Cytokeratin 14	285	Filamin C	357	HMGB3	429	LOXL1		
70	ARPP19	142	CD148	214	Cytokeratin 15	286	FKBP1A	358	HMGN2	430	LPA		
71	ART3	143	CD155	215	Cytokeratin 20	287	FKBP25	359	HNF-3 alpha	431	LSAMP		
72	ARTS1	144	CD157	216	Cytokeratin 9	288	FKBP51	360	hnRNP A1	432	LTBP4		

C. RayBio® Mouse Antibody Array L-3 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	AARE	73	Filaggrin	145	PABP1	217	PREP	289	RPL22	361	SIM2	433	TRAP1
2	ACAT1	74	FITM1	146	PACS1	218	PRG2	290	RPL23A	362	SIRPB1	434	TRAP220
3	ACOT2	75	GARS	147	PNLIP	219	PrP	291	RPL3	363	Six3	435	TRF 2
4	ADAM28	76	GCC2	148	PARVB	220	Profilin 1	292	RPL32	364	SLC4A1	436	TRIM14
5	AHCY	77	GLI-2	149	PCAP	221	Prolargin	293	RPL4	365	SLITRK1	437	Tropomyosin 3
6	AK1	78	GLOD4	150	PCBP1	222	Prosaposin	294	RPL7	366	SLURP1	438	TRP-1
7	AKR1A1	79	GLUL	151	PCBP2	223	PTGDS	295	RPL7A	367	SMAD6	439	TRPS1
8	ALDH2	80	GMPR1	152	PCCA	224	PSMD2	296	RPLP0	368	SMC4	440	Trypsinogen-2
9	DEFA5	81	GOLGA3	153	PCDH12	225	Protein C	297	RPLP2	369	SMPD4	441	TSR2
10	ANKRD9	82	GP2	154	PCDH8	226	Protein Z	298	RPS10	370	SNRPD1	442	TTC3
11	ANXA3	83	gp340	155	PCK2	227	PRR4	299	RPS11	371	SOD1	443	TTF1
12	AP180	84	GTF2F1	156	PCMT1	228	PRRC2A	300	RPS12	372	SOD2	444	TUBA6
13	AP3S2	85	HA1	157	PCNA	229	PRSS23	301	RPS13	373	SOD-3	445	TWF2
14	APLP2	86	HARS	158	PCPE-1	230	PRSS3	302	RPS14	374	Somatoliberin	446	TXNDC15
15	ApoA V	87	HIC1	159	PCSK9	231	PRTN3	303	RPS15A	375	Somatostatin	447	TXNDC4
16	ASPM	88	HIP55	160	PDAP1	232	PSMA1	304	RPS16	376	SORD	448	TXNDC5
17	ASS1	89	H1F0	161	PDE1B	233	PSMA2	305	RPS18	377	SorLA	449	TXNRD2
18	ATOX1	90	HIST1H1B	162	PDIA6	234	PSMA4	306	RPS19	378	SOX4	450	UBA1
19	ATPG	91	HIVEP2	163	PDLIM1	235	PSMA5	307	RPS2	379	SOX5	451	UBE2D3
20	AUTS2	92	hnRNP K	164	PDLIM3	236	PSMA6	308	RPS20	380	SP-D	452	Ube2L3
21	BAI2	93	hnRNP R	165	PDZD2	237	PSMB1	309	RPS23	381	Spectrin	453	UBE2N
22	BarX1	94	HNRNPUL2	166	PEBP1	238	PSMB2	310	RPS25	382	SPEN	454	UCH-L1
23	BBS1	95	HNRPA3	167	PEBP4	239	PSMB3	311	RPS3	383	SPG48	455	UFM 1
24	UBC9	96	HP1 gamma	168	PENK	240	PSMB4	312	RPS3A	384	SPINK5	456	UGGT
25	BLM	97	Importin 7	169	PEPD	241	PSMB5	313	RPS4X	385	SPS2L	457	CMPK1
26	BOLA2	98	Involucrin	170	perilipin-3	242	PSMB6	314	RPS5	386	SPTBN2	458	UNC13D
27	C10orf58	99	ISLR	171	Perilipin-1	243	PSMB7	315	RPS8	387	SPTLC1	459	UNC45A
28	CACNA1H	100	ITPR2	172	Periostin	244	PSMC3	316	RPS9	388	Src	460	UNC5H4
29	Calpain-2	101	ITPR3	173	Periplakin	245	PSMD1	317	RREB1	389	SSC5D	461	UPB1
30	CaMK2	102	KCNAB3	174	Peroxiredoxin-2	246	PSMD5	318	RSF1	390	STAT3	462	UQCRB
31	CaMK2D	103	LAMA5	175	Peroxiredoxin-3	247	PSMD9	319	RSU1	391	Stathmin 1	463	UQCRH
32	CBL	104	LDB3	176	Peroxiredoxin-1	248	PSME1	320	RUSC1	392	STI1	464	URB
33	CBR1	105	LHPP	177	PFAS	249	PSME2	321	Septin 7	393	STOM	465	URB2
34	CCDC58	106	LIPG	178	PFDN6	250	PTBP1	322	S100A1	394	STXBP2	466	UROC1
35	CCT6A	107	MAP4K4	179	PFKL	251	PTEN	323	S100A11	395	SUCLG1	467	UROD
36	CHCHD3	108	MICALL2	180	PGAM1	252	PTGR1	324	S100A7	396	SUMO3	468	Uroguanylin
37	Cingulin	109	MON2	181	PGAM2	253	PTK7	325	S100A9	397	SVEP1	469	URP2
38	CIT	110	MPST	182	PGK-1	254	PTMA	326	SDC4	398	Symplekin	470	USP14
39	CMG1	111	MRC2	183	PGLS	255	PTPRG	327	SAA4	399	SynCAM	471	USP2
40	CNBP	112	MSH3	184	PG-M	256	PTPRK	328	SBP-1	400	Synemin	472	USP5
41	CNPY2	113	MTA2	185	PGM1	257	PTPRM	329	SC35	401	SYNPO2L	473	Uteroglobin
42	Coilin	114	MTHFD1	186	PGRPL	258	PTPRZ	330	SCG	402	Syntaxin 7	474	Utrophin
43	COL8A2	115	MUC5B	187	PHGDH	259	PZP	331	SCN3A	403	TAB182	475	VARS
44	COLEC11	116	MVD	188	Piccolo	260	QARS	332	SCP2	404	Talin1	476	VAP-1
45	COPG2	117	Myosin IIB	189	pIgR	261	QDPR	333	SDNSF	405	TARS	477	VAP-A
46	CORO1B	118	NACA1	190	PIK3C2B	262	QPRT	334	SDPR	406	TAX1BP3	478	VCP
47	CPA3	119	NAGPA	191	PIN	263	Quiescin Q6	335	SECISBP2	407	TBCA	479	VDAC1
48	CPI17 alpha	120	NAV2	192	PIP5K2 alpha	264	Rab1A	336	Secretogranin V	408	TCEB2	480	VILIP3
49	CrkRS	121	NFATC4	193	PISD	265	Rab7a	337	Semaphorin 6B	409	Tcf20	481	Vimentin
50	CRLF3	122	NNT	194	PLA2G1B	266	Ran	338	Semaphorin 7A	410	TCP1 delta	482	VNN1
51	CSRP3	123	NPEPPS	195	Plastin 3	267	RanBP1	339	SERBP1	411	TCP1 eta	483	VPS4B
52	CTNNAL1	124	NQO2	196	Plastin L	268	RanGAP1	340	Serpin A11	412	TCP1 theta	484	VSIG4
53	CTNND1	125	NSFL1C	197	PLBD2	269	RAP1B	341	Serpin A7	413	TCTP	485	WDR1
54	Cyclophilin F	126	NUCB1	198	PLD4	270	Rbm15	342	Serpin B3D	414	TDIF2	486	WDR44
55	Cystatin C	127	NUP214	199	Plectin	271	RCL	343	Serpin B6	415	Tenascin C	487	WISP2
56	DCAMKL1	128	OAF	200	Plexin B1	272	RECQ4	344	Serpin B8	416	Tenascin XB	488	WNK2
57	Dematin	129	OIT3	201	Plexin B2	273	Reg3A	345	Serpin F2	417	TFF2	489	XPG
58	DIAPH1	130	OPCML	202	PLOD1	274	REV3L	346	Serpin H1	418	TGM3	490	YB1
59	DKC1	131	ORM2	203	PLOD2	275	RHOC	347	Serpin A10	419	Thioredoxin-1	491	SYN1
60	DLST	132	OSBP1	204	Plxdc2	276	RHOG	348	SERPINB1	420	THOP1	492	YY1
61	DMRT1	133	OSCAR	205	PMCA	277	RNASE1	349	SerpinB4	421	TIF1 alpha	493	ZAK
62	Dystrophin	134	OSM R beta	206	PNP	278	RNASET2A	350	SerpinE2	422	TMEM103	494	zbtb11
63	Ebf4	135	Osteoadherin	207	POLD2	279	RLF	351	SerRS	423	TOB2	495	ZBTB4
64	EBP50	136	OTC	208	POLR2A	280	RNASE4	352	SET	424	TOMM70A	496	ZC3H18
65	ECHDC1	137	OTUB1	209	POR	281	Rnose2	353	SEZ6L2	425	TOP2B	497	ZC3H4
66	EHHADH	138	OTUD7A	210	PPOX	282	RP1	354	SF20	426	TPD52L2	498	ZC3H8
67	EIF3D	139	OT-NPI	211	PPP1CC	283	RPL10	355	SHANK1	427	TPM4	499	ZNF295
68	eIF4A2	140	p16 ARC	212	PPP1R9A	284	RPL10A	356	SHC1	428	TPP1	500	Zyxin
69	eIF4GII	141	p23	213	PPP2R1B	285	RPL11	357	SHMT1	429	TPPP3		
70	ENDOD1	142	p39	214	PPP2R4	286	RPL12	358	SHOX	430	TPR		
71	EYA2	143	P4HB	215	PRCP	287	RPL14	359	SHP-1	431	TALDO1		
72	F8	144	p73	216	PRDM13	288	RPL17	360	Siglec-1	432	Transthyretin		

VII. Interpretation of Results:

A. Explanation of Controls Spots

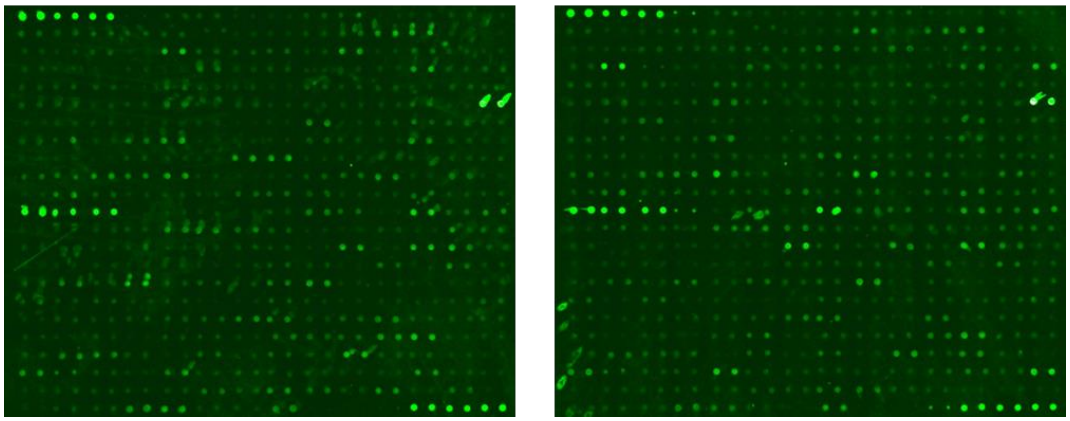
There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized biotinylated IgG. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal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

B. Typical Results

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

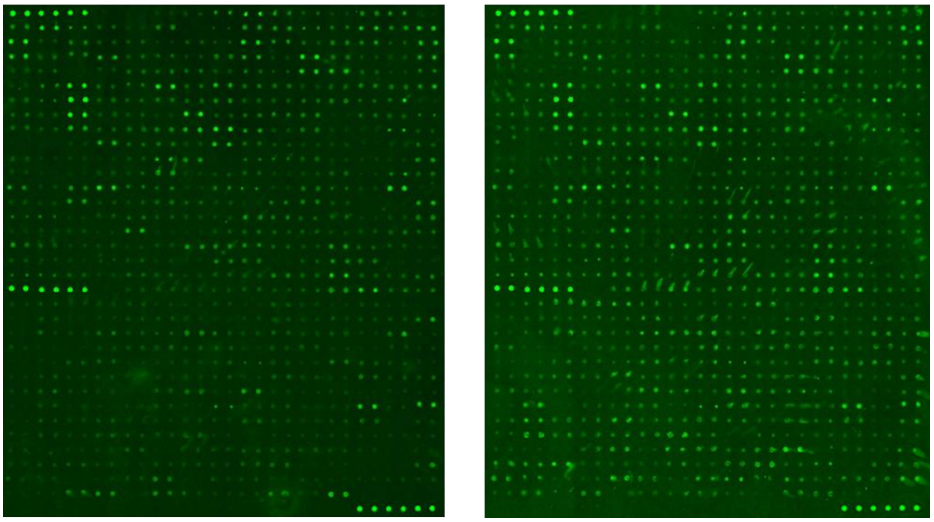
RayBio® Mouse Antibody Array L-308

Serum Plasma



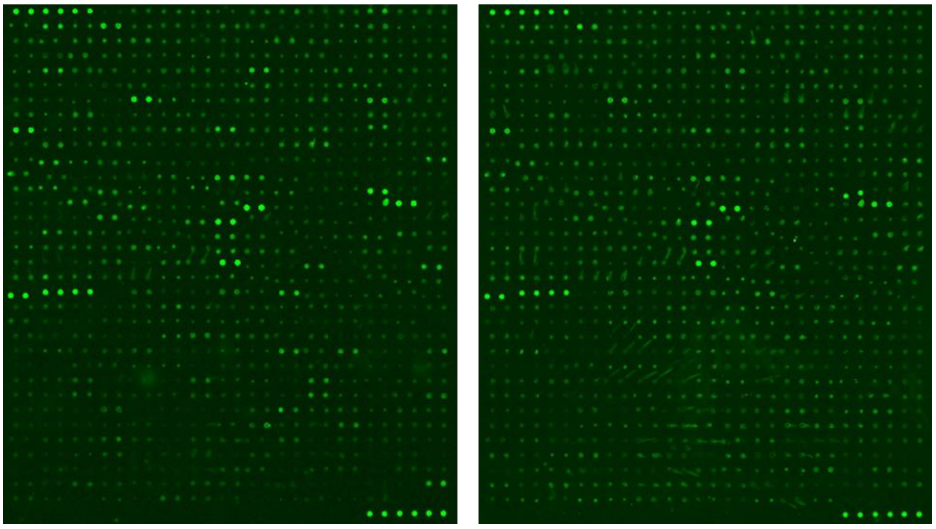
RayBio® Mouse Antibody Array L-2

Serum Plasma



RayBio® Mouse Antibody Array L-3

Serum Plasma



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

C. Background Subtraction

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

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

D. Normalization of Array Data

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary. For example, in our Analysis Tool Software (described below), the array represented by data entered in the left-most column each worksheet is the default "reference array."

You can calculate the normalized values as follows:

$X(Ny) = X(y) * P1/P(y)$

Where:
P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is freely available for use with data obtained using RayBio® Biotin Label-based Antibody Arrays. You can copy and paste your signal intensity data (with and without background) into

the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls. Analysis Tool software can be downloaded from the product page on the RayBiotech website.

E. Threshold of Significant Difference

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences in expression levels of each protein between samples or groups.

Any ≥1.5-fold increase or ≤0.65-fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy ≈ 95%).

F. Pathway Analysis of the Array Proteins

Mouse antibody array L-1308 detects 1308 unique mouse proteins, including most analyzed cytokines, chemokines, adipokines, extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins. All the array proteins are provided with their Uniprot number and GeneID, which are essential for further data mining. Raybiotech offers affordable biostatistics and bioinformatics service, including data clean-up, differential expression analysis, cluster analysis, biomarker selection, pathway analysis and experimental design. See more details on the website: https://www.raybiotech.com/biostatistics-and-bioinformatics-services

VIII. Troubleshooting Guide

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

IX. Selected References

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 Rats*. Journal of Proteome Research. 2010, 9, 5228–5238

Chowdury UR, Madden BJ, Charlesworth MC, Fautsch MP., *Proteomic Analysis of Rat 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: relationship 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

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

This product is for research use only.