RayBio® Label-Based (L-Series) Mouse Antibody Array L-2

Patent Pending Technology

User Manual (revised Dec 9, 2019)

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

L-Series Mouse Antibody Array L-2
Cat# AAM-BLG-2-4 (4 Sample Kit)
Cat# AAM-BLG-2-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

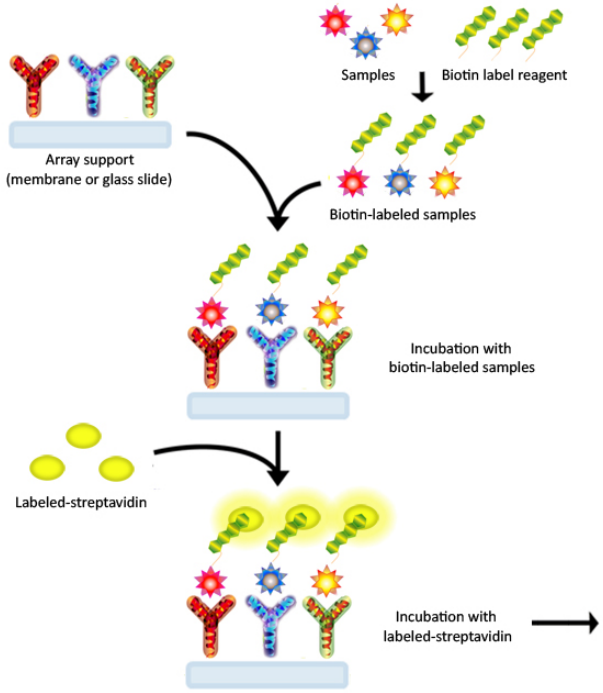
Tel: (Toll Free) 1-888-494-8555 or +1-770-729-2992; Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

TABLE OF CONTENTS

I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the L-Series Mouse Antibody Array L-2, researchers can now obtain a broad, panoramic view of protein expression. The expression levels of 500 mouse target proteins can be simultaneously detected, including extracellular matrix proteins, growth factors, angiogenic factors, proteases, enzymes, soluble and transmembrane receptors and transport proteins, adhesion molecules and other proteins in cell culture supernatants, cell lysate, tissue lysate, serum and plasma.

The first step in using the RayBio® L-Series Mouse Antibody Array L-2 is to biotinylate the primary amine groups of the proteins in your sample (sera or plasma, cell culture supernatants, cell lysates or tissue lysates). The glass slide arrays are then blocked, just like a Western blot, and the biotin-labeled sample is added onto the glass slide, which is pre-printed with capture antibodies. The slide is incubated to allow binding of target proteins. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then applied to the array. Finally, the glass slide is dried, and laser fluorescence scanning is used to visualize the signals.



II. Materials Provided

A. Storage Recommendations

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

ITEM	DESCRIPTION	AAM-BLG-2-4 (L-2)	AAH-BLM-2-8 (L-2)
A	Dialysis Vials & Floating Dialysis Rack	8 vials	16 vials
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio® L-Series Mouse Antibody Array L-2 Glass Slides*	1 slide (L-2)	2 slides (L-2)
F	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml)
G	20X Wash Buffer I	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer II	1 bottle (30 ml)	1 bottle (30 ml)
I	Cy3-Conjugated Streptavidin	1 vial	2 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (8 ml)	
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	
M	30 ml Centrifuge Tube	1 tube	

*Each slide contains 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.

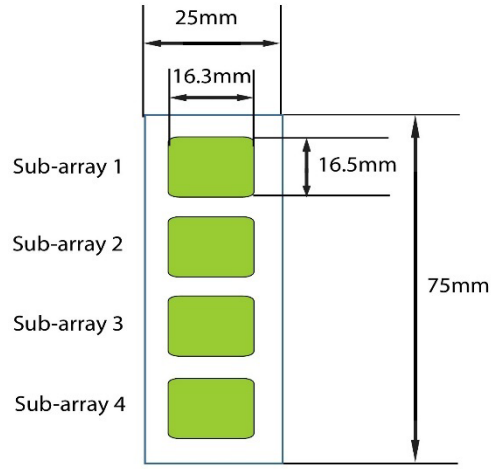
- Handle the slides with powder-free gloves and in a clean environment.

- Do not remove the glass slide from the chamber assembly until step 20 on page 16, 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 Mouse L-2 Glass 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.

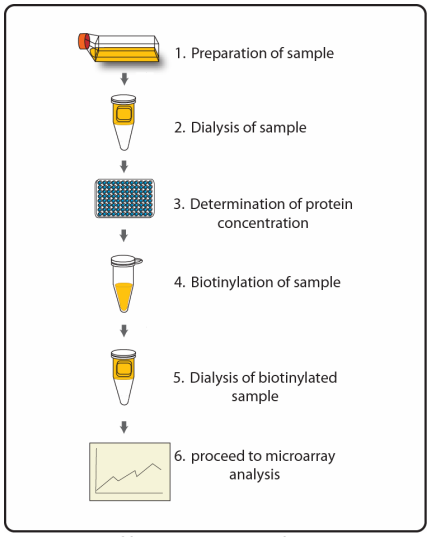
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.

- Unlike most Cy3 fluors, streptavidin-conjugated fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from directly strong light and temperatures above RT.

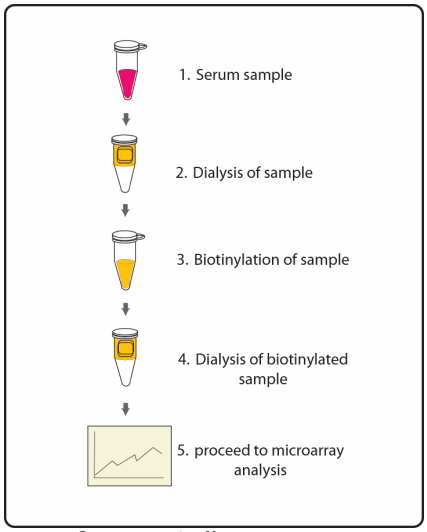
IV. Protocol

Assay Diagram

1. Cell culture supernatants or cell/tissue lysates



2. Serum or plasma



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

A. Dialysis of Sample

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

1. Prepare enough dialysis buffer (1X PBS, pH=8.0) for all dialysis steps herein and after. To prepare 1 L dialysis buffer, dissolve 0.2 g KCl, 8 g NaCl, 0.2 g KH_2PO_4 and 1.15 g Na_2HPO_4 in 800 ml ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 ml with ddH_2O.
2. Add each sample into a separate Dialysis Tube (Item A). Loading volumes are as follows: 200 µl cell culture supernatant; 100 µl cell or tissue lysate (1~2 mg/ml total protein); 20 µl serum or plasma + 80 µl dialysis buffer (5-fold dilution). Carefully place Dialysis Tubes into Floating Dialysis Rack.

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

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

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

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

B. Biotin-labeling Sample

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

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

5. Add 1X Labeling Reagent to dialyzed samples.

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

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

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

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

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

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

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

C. Drying the Glass Slide

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

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

Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubations

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

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

11. Immediately prior to sample incubation, spin biotin-labeled samples for 5 min at 10,000 rpm to remove any particulates or precipitates. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 2-10-fold for cell culture supernatants, 20-fold for serum/plasma and 30-fold for cell/tissue lysate.

Note: Optimal sample dilution factor will depend on the abundance of target proteins. If the background or antigen-specific antibody signals are too

strong, the sample can be diluted further in subsequent experiments. If the signal is too weak, more concentrated samples can be used.

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

Note: Avoid the flow of sample into neighboring wells.

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

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

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

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

17. Prepare 1X Cy3-Conjugated Streptavidin:

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

    b) Add 1000 µl of Blocking Buffer into the tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do 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.

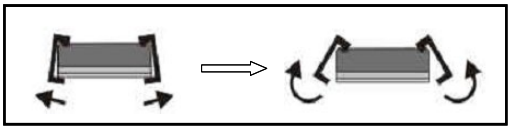
19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 2 hours with gentle rocking or shaking.

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.

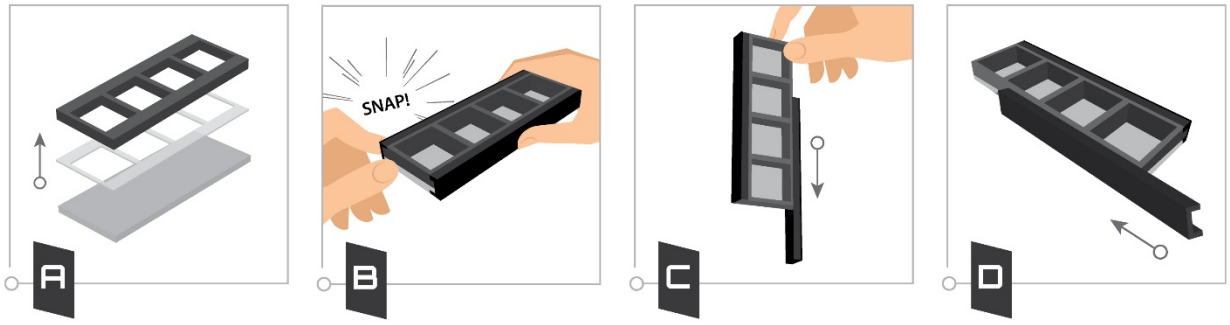
E. Fluorescence Detection

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

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

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

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



V. Antibody Array Map and Target List

A. RayBio® Mouse Antibody Array L-2 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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
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
436	436	437	437	438	438	439	439	440	440	441	441	442	442	443	443	444	444	445	445	446	446	447	447	448	448	449	449	450	450
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
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
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
496	496	497	497	498	498	499	499	500	500	501	501	502	502	503	503	504	504	505	505	506	506	507	507	508	508	neg	neg	neg	neg
neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	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® Mouse Antibody Array L-2 Target List

number	name	number	name	number	name	number	name	number	name
1	Pos 1a	61	Annexin A1	121	Cadherin-6	181	COG4	241	DRIL1
2	Pos 2a	62	Annexin A2	122	CALD1	182	COL19A1	242	DSCAM
3	Pos 3a	63	Annexin A5	123	Calpain S1	183	COL4A3	243	DSPG3
4	Neg	64	Annexin A6	124	Calpastatin	184	Col6A2	244	ECHS1
5	14-3-3 beta	65	ANP	125	Calponin-2	185	COL9A3	245	ECI1
6	14-3-3 zeta	66	ANP32A	126	Calretinin	186	COLEC10	246	ECM1
7	53BP1	67	Antithrombin III	127	Calumenin	187	Collagen I a1	247	EEF1G
8	aAmylase	68	APLP1	128	CAP1	188	Collagen III	248	EEF2
9	AAT1	69	AQR	129	CAPZA1	189	Collagen IVa6	249	EFEMP2
10	ABAT	70	ARFGEF3	130	Carbonic anhydrase 2	190	Collagen IX	250	EFTUD2
11	ABCF1	71	Arp3	131	Carbonic anhydrase 3	191	Collagen V	251	EHD3
12	ABI3BP	72	ARPC2	132	Caspase-14	192	Collagen X	252	Eif4a1
13	ACAA1	73	ARPC3	133	Catalase	193	Collagen XV	253	ELAVL1
14	ACAA2	74	ARPP19	134	Cathelicidin	194	COMP	254	EMSY
15	ACACA	75	ART3	135	Cathepsin A	195	Corneodesmosin	255	EN2
16	ACLY	76	ARTS1	136	Cathepsin G	196	Cortactin	256	Endorepellin
17	ACO1	77	ASGR2	137	Cathepsin H	197	COTL1	257	ENO3
18	ACTBL2	78	ASH2L	138	Cathepsin Z	198	CPB2	258	ENSA
19	ACTC1	79	ASL	139	CBS	199	CPE	259	EPB41
20	ACTG1	80	Aspartate Aminotransferase	140	CCAR2	200	CPEB3	260	EPCR
21	ACTG2	81	Aspartyl Aminopeptidase	141	CCDC126	201	CPM	261	Ephrin B1
22	ACTN1	82	ASXL1	142	CCDC25	202	CPNE3	262	Eps 15
23	ADA	83	ATP5A1	143	CCS	203	CRHBP	263	ERAB
24	ADAMDEC1	84	ATPB	144	CD109	204	CrkL(1)	264	ERp29
25	ADAS	85	B3GNT2	145	CD133	205	CRMP2	265	ERp57
26	ADGRF5	86	B4GalT1	146	CD148	206	CRTAC1	266	ERp72
27	ADGRL4	87	B7-H2	147	CD155	207	CRYZ	267	ESD
28	ADH1	88	BAD	148	CD157	208	Cyclophilin A	268	ESR1
29	ADH1C	89	BASP1	149	CD21	209	Cyclophilin B	269	Ezrin
30	ADH4	90	Bassoon	150	CD39L4	210	Cystatin	270	FABP5
31	ADH5	91	Bcl2l2	151	CD41	211	CYTL1	271	Factor IX
32	ADM	92	BCoR	152	CD42b	212	Cytochrome b5	272	Factor V
33	Advillin	93	beta I Spectrin	153	CD48	213	Cytochrome c	273	Factor XI
34	AEBP1	94	beta I Tubulin	154	CD5L	214	Cytokeratin 1	274	Factor XII
35	AFG3L2	95	beta III Tubulin	155	CD98	215	Cytokeratin 10	275	Factor XIII
36	AGA	96	BID	156	CDA	216	Cytokeratin 13	276	FAH
37	Aggrecan	97	BIN2	157	CDK2	217	Cytokeratin 14	277	FAM20C
38	Agrin	98	Biotinidase	158	CED-6	218	Cytokeratin 15	278	FAM3C
39	AGXT	99	BIRC6	159	CENPF	219	Cytokeratin 20	279	FASN
40	Ahsp	100	BMP-1	160	CEP57	220	Cytokeratin 9	280	FASTKD5
41	AIFM1	101	BPGM	161	CES1	221	D4	281	FBP 38
42	AKAP9	102	BPIFB1	162	Cezanne	222	DAN	282	FDPS
43	AKR1B1	103	BPIFB2	163	CFB	223	DARS2	283	FGG
44	AKR7A2	104	Brevican	164	CFHR1	224	DBH	284	Fibrillin 1
45	ALAD	105	BRG1	165	CFI	225	DCXR	285	Fibrinogen-like 2
46	ALDH16A1	106	BRSK1	166	CFVII	226	DDAH1	286	Pos 1b
47	ALDH1A1	107	C1QA	167	Chitobiase	227	DDT	287	Pos 2b
48	ALDH9A1	108	C1QB	168	Chitotriosidase(1)	228	DDX3Y	288	Pos 3b
49	alpha Actinin 4	109	C1QR	169	Cholinesterase	229	DEFA6	289	Neg
50	alpha Synuclein	110	C1RL	170	CHORDC1	230	Desmocollin 1	290	Fibrinopeptide B
51	alpha Tubulin 4	111	C1s	171	CHREBP	231	Desmocollin-2	291	Fibulin 3
52	ALPL	112	C4BPA	172	Chromogranin B	232	Desmocollin-3	292	Ficolin 2
53	ALS	113	C6	173	CKB	233	Desmoglein-1	293	Filamin C
54	Alsin	114	C8A	174	CLIC1	234	Desmoglein-2	294	FKBP1A
55	Aminoacylase 1	115	C8G	175	CLIP1	235	Desmoplakin 3	295	FKBP25
56	Aminopeptidase A	116	C9orf40	176	CL-P1	236	DGK-theta	296	FKBP51
57	Androgen Receptor	117	CA1	177	CLTA	237	DISC 1	297	Fodrin alpha chain
58	ANGPTL6	118	CA150	178	CNOT1	238	DMRN9	298	Frizzled 8
59	ANGPTL8	119	CACNB4	179	CO4A2	239	DOT1L	299	FRY
60	Ankrd26	120	Cadherin 22	180	Cofilin-1	240	DPP3	300	FSH-B

RayBio® Mouse Antibody Array L-2 Target List…Continued

number	name	number	name	number	name	number	name
301	FTL1	361	Histone H3.3	421	Lamin A/C	481	MYL12B
302	FUCA2	362	Histone H4	422	Lamin B2	482	MYO5A
303	FUS	363	HMGB1	423	Laminin A2	483	Myoferlin
304	G3BP1	364	HMGB2	424	Laminin b2	484	Myosin 18B
305	G6PD	365	HMGB3	425	Laminin gamma 1	485	Myosin9
306	GALNT2	366	HMGN2	426	LAMP1	486	NABC1
307	GANAB	367	HNF-3 alpha	427	LASP1	487	NAGLU
308	GAPDH	368	hnRNP A1	428	LCAT	488	NAP1L1
309	GARNL1	369	hnRNP A2B1	429	LCMT2	489	NAPRT1
310	GART	370	hnRNP C1 + C2	430	LDH-H	490	NASP
311	Gastrokine 1	371	hnRNP G	431	LEDGF	491	NCAM2
312	GATM	372	hnRNP L	432	Limbin	492	Nebulin
313	GBE1	373	hnRNP M	433	LIMS1	493	Nectin-1
314	GCDFP 15	374	hnRNP U	434	LMW-PTP	494	Nectin-3
315	GCLC	375	Hornerin	435	LOK	495	Neogenin
316	GCSH	376	Hoxb3	436	LOX	496	Nesprin2
317	GDA	377	HOXD11	437	LOXL1	497	Neurofibromin
318	GDF7	378	HP1BP3	438	LPA	498	Neurogranin
319	GDI1	379	HPD	439	LSAMP	499	Neuropeptide B
320	GDI2	380	HPRT1	440	LTBP4	500	Neuropilin-1
321	Gephyrin	381	HRG	441	Lubricin	501	Neurotrimin
322	GFAP	382	HRP12	442	LUZP1	502	NF-M
323	GGCT	383	HSPA1A	443	LYZL1	503	NIF3L1
324	GGH	384	HTRA1	444	MAGI2	504	NME3
325	GIP	385	HUWE1	445	MAN1	505	nNOS1
326	GLIPR2	386	IDH1	446	MAN1A1	506	Notch-2
327	GLUD1	387	IFRD1	447	Mannosidase II	507	NPAS3
328	Glycoprotein V	388	IGF2BP2	448	MAP1A	508	NPM1
329	GM2A	389	IGFBP7	449	MAPRE1	509	Neg
330	GMF beta	390	IGSF4B	450	MARCKS	510	Neg
331	GNB1	391	ILK	451	MASP3	511	Neg
332	GNPTG	392	Inhibin beta	452	MBD2	512	Neg
333	GOLIM4	393	Integrin b1	453	MBP	513	Neg
334	GOLM1	394	Integrin beta 6	454	MCAM	514	Neg
335	GPD1	395	Intergrin a6	455	Mcl-1	515	Neg
336	GPLD1	396	IQGAP2	456	MCM	516	Neg
337	GRHPR	397	IRE1	457	MDH1	517	Neg
338	GRP170	398	IRS2	458	MEP1A	518	Neg
339	GSS	399	ISOC2	459	Metallothionein 2	519	Neg
340	GSTM1	400	ITGB4BP	460	Metavinculin	520	Neg
341	GSTO1	401	ITIH2	461	MFAP4	521	Neg
342	GSTP1	402	ITIH3	462	MFI2	522	Neg
343	Guanylin	403	ITIHC4	463	mGLUR5	523	Pos 1c
344	GZMM	404	JAM-A	464	Mimecan	524	Pos 2c
345	H6PD	405	JPT1	465	MLCK	525	Pos 3c
346	HABP2	406	KDM4B	466	MMR	526	
347	HBB	407	Keratin 36	467	MN1	527	
348	HDGF	408	KIAA0319L	468	Moesin	528	
349	Hemoglobin	409	KIAA1468	469	MP1	529	
350	Hemoglobin A1c	410	KLKB1	470	MPCA	530	
351	HEXB	411	KMT2D	471	MPO	531	
352	HGFA	412	KRT31	472	MRP 1	532	
353	HIBADH	413	KRT33B	473	MSH6	533	
354	HINT1	414	KRT73	474	Mtor	534	
355	HIP1R	415	KRT82	475	Multimerin 2	535	
356	Histone H1.2	416	KRT85 - N-terminal	476	MyBPC3	536	
357	Histone H1.4	417	KSR1	477	MYH2	537	
358	Histone H2A	418	LAF4	478	MYH6	538	
359	Histone H2A.Z	419	LAIR1	479	MYH7	539	
360	Histone H2B K	420	LAM b1	480	MYHC2x	540	

VI. Interpretation of Results:

A. Explanation of Controls Spots

1) Positive Control spots (POS1, POS2, POS3) are standardized amounts of biotinylated IgG 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.

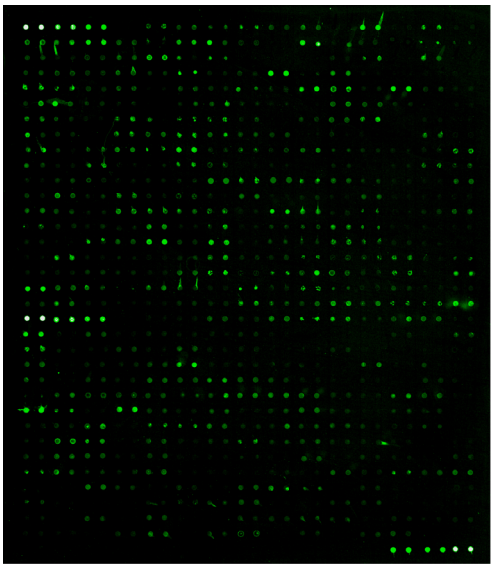
2) Negative Control (NEG) spots contain a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of the Cy3-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical Results

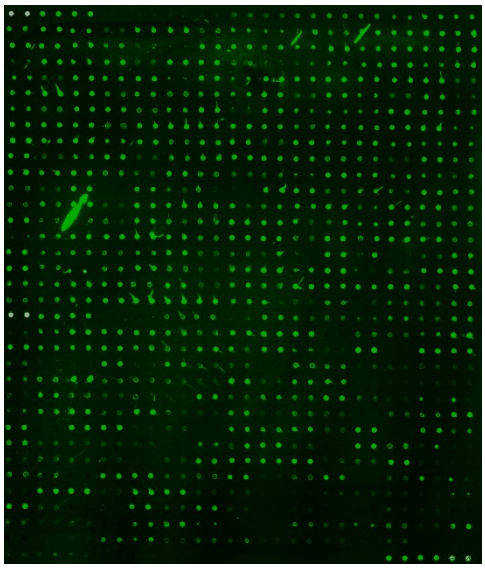
The following figure shows the RayBio® L-Series Mouse Antibody Array L-2 probed with a serum sample. The images were captured using an Axon GenePix laser scanner. The strong signals in row 20 and the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays.

RayBio® L-Series Mouse Antibody Array L-2

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 (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 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.