RayBio® Label-based (L-Series) Human Obesity Antibody Array 1 Membrane Kit

Patent Pending Technology
User Manual (Revised October 30, 2018)

For the simultaneous detection of the relative expression of 182 human proteins in serum, plasma, and cell/tissue lysates.

Cat# AAH-BLM-ADI-1B-2 (2 Sample Kit)
Cat# AAH-BLM-ADI-1B-4 (4 Sample Kit)

Please read manual carefully
before starting experiment

Your Provider for Excellent Protein Array Systems and Services

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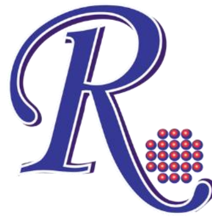

RayBiotech, Inc

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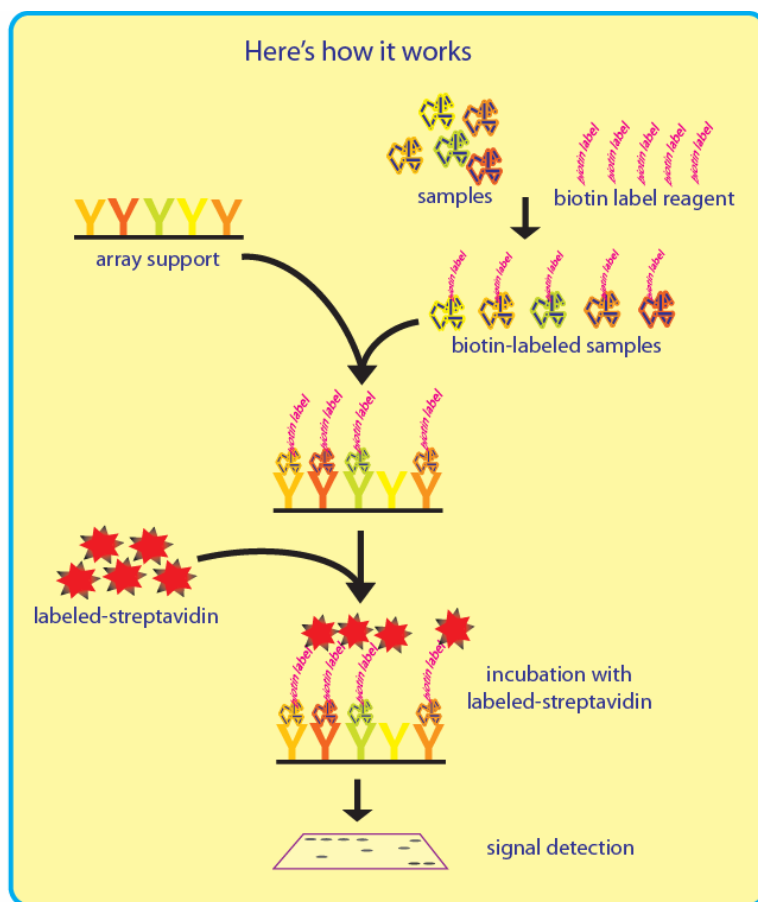
I. Introduction

Recent technological advances by RayBiotech have enabled the largest commercially available antibody array to date. With the RayBio® L-Series Human Obesity Antibody Array 1, researchers can now obtain a broad, panoramic view of cytokine expression. The expression levels of 182 human proteins can be simultaneously detected, including cytokines, chemokines, adipokine, growth factors, angiogenic factors, proteases, soluble receptors, soluble adhesion molecules and other proteins in cell tissue lysates and serum/plasma.

The first step in using the RayBio® L-Series Human Obesity Antibody Array 1 is to biotinylate the primary amine of the proteins in the sample. The membrane arrays are then blocked, similar to a Western blot, and the biotin-labeled sample is added onto the membrane array which is pre-printed with capture antibodies and incubated to allow for interaction of target proteins. After incubation with HRP-Conjugated Streptavidin, the signals can be visualized by chemiluminescence.



II. Materials Provided

A. Storage Recommendations

Upon receipt, Box 1 should be stored at -20 °C and Box 2 should be stored at 4 °C. The kit must be used within 6 months from the date of shipment. After initial use, Blocking Buffer, Stop Solution, HRP-Conjugated Streptavidin, Detection Buffers C and D should be stored at 4 °C to avoid repeated freeze-thaw cycles (may be stored

for up to 3 months, Labeling Reagent, Item B should be fresh preparation before use). The Array Membrane should be kept at -20 °C and avoid repeated freeze-thaw cycles (may be stored for up to 6 months).

Box 1 (store at -20 °C):

ITEM	DESCRIPTION	AAH-BLM-ADI-1B-2	AAH-BLM-ADI-1B-4
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	
E	RayBio L-Series Human Obesity Antibody Array 1 membranes	2 membranes Human Obesity Antibody Array 1	4 membranes Human Obesity Antibody Array 1
F	Blocking Buffer	1 vials (30 ml/ea)	2 vials (30 ml/ea)
I	500X HRP-Conjugated Streptavidin Concentrate	1 vial (100 µl)	2 vials (100 µl/ea)
K	Detection Buffer C	1 vial (10 ml)	2 vials (10 ml/ea)
L	Detection Buffer D	1 vial (10 ml)	2 vials (10 ml/ea)
Other Kit Components: Plastic Sheets			

Box 2 (store at 4 °C):

ITEM	DESCRIPTION	AAH-BLM-ADI-1B-2	AAH-BLM-ADI-1B-4
A-2	Dialysis Vials	2 vials	4 vials
G	20X Wash Buffer 1 Concentrate	1 vial (30 ml)	1 vial (30 ml)
H	20X Wash Buffer 2 Concentrate	1 vial (30 ml)	1 vial (30 ml)
J-2	Spin Columns	2 columns	4 columns
N/A	Plastic Incubation Trays (w/lid)	2 trays	4 trays
M	Floating Dialysis Rack	1 rack	

B. Additional Materials Required

- 1X PBS, pH=8.0
- Shaker
- 2 - 5 ml tube
- 50 ml conical collection tubes
- Distilled water
- Kodak X-Omat™ AR film (REF 165 1454) and film processor or Chemiluminescence imaging system
- Large beaker
- Stir plate
- Eppendorf tube

III. Overview and General Considerations

A. Handling Array Membranes

- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

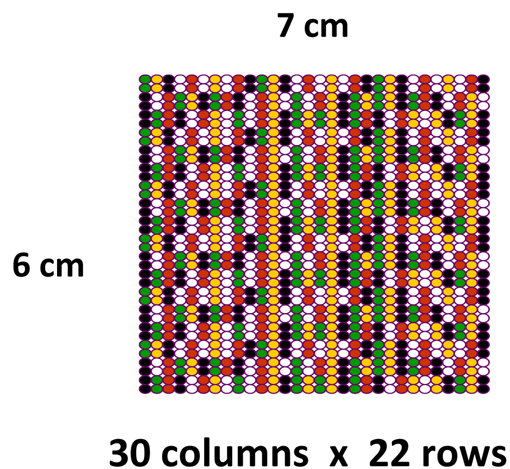
B. Incubation

- Completely cover membranes with sample or buffer during incubation and cover Plastic Incubation Tray with lid to avoid drying.
- Avoid foaming during incubation steps.

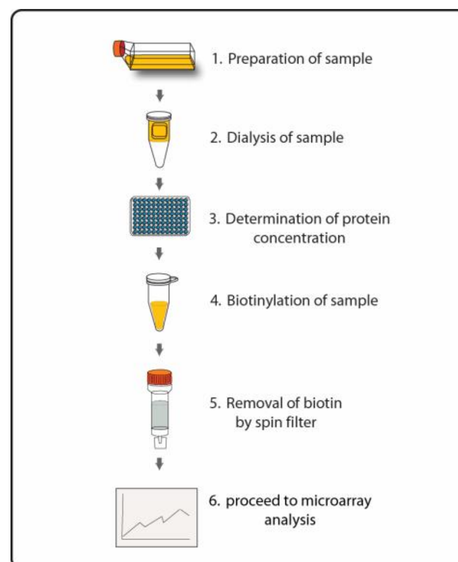
- Perform all incubation and wash steps under gentle rotation.
- Several incubation steps such as step 3 in page 10 (sample incubation) or step 7 in page 11 (HRP-Conjugated Streptavidin incubation) may be done at 4 °C for overnight.

IV. Protocol

Layout of Human Obesity Antibody Array 1 Membrane



Assay Diagram



A. Preparation of Samples

1. Extracting Protein from Cells

1) Centrifuge cells.
   a. Adherent cells:
      i. Remove supernatant from the cell culture and wash cells gently two times with cold 1X PBS taking care not to disturb the 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:
      i. Pellet the cells by centrifuging using a microcentrifuge tube 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 deionized or distilled water). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.

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

   Note: If the supernatant appears to be cloudy, transfer the supernatant 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 -80°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. Determine the cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at – 80°C.

2. Extracting Protein from Crude Tissue

1) Transfer approximately 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water).

2) Homogenize the tissue according to the homogenizer manufacturer's 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 supernatant 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 -80°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4) Transfer the supernatant to a clean tube and store at – 80°C.

B. Dialysis of Sample

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

1. Prepare dialysis buffer (1X PBS) by dissolving 0.6 g KCl, 24 g NaCl, 0.6 g KH_2PO_4 and 3.45 g Na_2HPO_4 in 2500 ml de-ionized or distilled water. Adjust to a pH of 8.0 with 1M NaOH and adjust final volume to 3000 ml with de-ionized or distilled water.

2. Load each sample into a separate Dialysis Vials (Item A-2), Loading volumes are as follows: 0.1-0.2 ml cell or tissue lysate (~ 1-2 mg/ml total protein) per vial for dialyzing; 30 µl serum or plasma + 120 µl dialysis buffer (5-fold dilution) per vial for dialyzing. Carefully place all Dialysis Vials into the Floating Rack.

3. Place the Floating 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, occasionally gently stirring the dialysis buffer. Then exchange the dialysis buffer with fresh buffer and repeat dialysis for at least 3 hours at 4 ˚C. Transfer dialyzed samples into a clean eppendorf tube. Centrifuge dialyzed samples for 5 minutes at 10,000 rpm to remove any particulates or precipitates and then transfer and combine each sample into one clean eppendorf tube. Mix well by gently pipetting.

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

Note: Determine the total protein concentration for cell or tissue lysates after the dialysis procedure (Step 3). RayBiotech recommends a BCA total protein assay (e.g. Pierce BCA Protein Assay Kit, cat# 23227).

C. Biotin-labeling of Sample

Avoid contamination with any solution containing amines (i.e., Tris, glycine) as well as azides during the biotinylation process.

4. Immediately before use, prepare 1X Labeling Reagent by briefly centrifuging down the Labeling Reagent vial (Item B) and add 500 µl 1X PBS (pH=8.0) into the vial. Pipette up and down or vortex briefly to dissolve the powder.

5. Add an appropriate amount* of 1X Labeling Reagent into the tube containing the sample and immediately mix the reaction solution. Incubate the reaction solution at room temperature for 30 minutes with gentle shaking. Gently tap the tube to mix the reaction solution every 5 minutes.

    a. For labeling cell or tissue lysates: transfer 90 µg total protein (e.g., 45 µl of 2 mg/ml) cell or tissue lysates into a tube and add enough 1X PBS for a total volume of 260 µl. Then add 10 µl 1X Labeling Reagent.

b. For labeling serum or plasma: Add 65 µl of 1X Labeling Reagent Solution into a new tube containing 105 µl dialyzed serum or plasma sample and 80 µl 1X PBS.

Note: To normalize serum/plasma concentrations during biotinylation, measure the sample volume before and after dialysis. Then adjust the volumes of the dialyzed serum/plasma and 1X PBS Buffer to compensate. For example, if the sample volume increases 1.4 fold (from 150 µl to *210 µl, see "Dialysis of Sample" on page 11) after dialysis, then use 1.4 fold more serum/plasma (1.4*105 µl = 147 µl) and reduce the added 1X PBS to 38 µl (80 µl - 42 µl = 38 µl).*

6. Add 5 µl Stop Solution (Item D) into the reaction solution and then use the Spin Column (Item J-2) to remove any unbound biotin.

a). Twist off the bottom closure of the Spin Column and loosen the cap (but keep the cap on). Place the Spin Column into a 50 ml conical collection tube.

b). Centrifuge the Spin Column at 1,000 g for 3 minutes to remove storage solution.

Note: The resin should appear compacted after centrifugation.

c). Add 5 ml 1X PBS (pH=8.0) into the Spin Column and centrifuge at 1,000 x g for 3 minutes to remove the 1X PBS. Repeat an additional 2 times to wash the Spin Column.

d). Place the Spin Column in a new 50 ml conical collection tube and slowly load 3.5 ml of sample to the center of the compact resin bed.

Note: The maximal sample volume is 700 ul for J-2 each Spin Column. Do not load over maximal sample volume sample into a Spin Column.

e). Centrifuge the Spin Column at 1,000 x g for 3 minutes. The sample should filter through the resin and deposit into the 50 ml conical collection tube. Store at -80 ^{0}C until needed. Discard the Spin Column after use.

D. Blocking and Incubation

7. Place each membrane printed side up into a Plastic Incubation Tray (provided). 1 membrane per tray.

   Note: The printed membrane will have a "-" mark in the upper left corner of the membrane.

8. Add 8 ml of Blocking Buffer (Item F) to each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour.

9. Aspirate Blocking Buffer from each tray. Add 8 ml of diluted* or undiluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

Note: 1). It is recommended to use 8 ml of 80-fold diluted biotin-labeled cell/tissue lysate or 8 ml 70 fold diluted biotin-labeled serum/plasma. Dilute sample using Blocking Buffer.

Note: 2). The concentration of sample used depends on the abundance of proteins. The samples can be concentrated if the overall signals are too weak. If the overall signals are too strong, the sample can be diluted further.

Note: 3). Incubation may be done at room temperature with gentle shaking for 2 hours or overnight at 4°C.

10. Dilute 20X Wash Buffer 1 with deionized or distilled water to prepare the 1X Wash Buffer 1. Aspirate the samples from each tray and then wash by adding 20 ml of 1X Wash Buffer I at room temperature with gentle shaking (5 min per wash). Repeat the wash 2 more times for a total of 3 washes.

11. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 with deionized or distilled water to prepare the 1X Wash Buffer 2. Wash 3 times with 20 ml of 1X Wash Buffer 2 at room temperature with gentle shaking.

12. Aspirate the 1X Wash Buffer 2 from each tray. Dilute the 500X HRP-Conjugated Streptavidin with Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. Add 8 ml of 1X HRP-Conjugated Streptavidin to each membrane.

Note: Ensure that the vial containing the 500X HRP-Conjugated Streptavidin is mixed well before use, as precipitation can form during storage.

13. Incubate at room temperature with gentle shaking for 2 hours.

Note: incubation may be done at 4 ^{0}C for overnight.

14. Wash as directed in steps 10 and 11.

E. Detection

* Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.

15. For detection of 2 membranes, add 4.2 ml of Detection Buffer C and 4.2 ml of Detection buffer D into a tube and mix both solutions. Drain off excess wash buffer. Place membrane antibody side up ("-" symbol is marked in the top left corner of each membrane) on a clean plastic plate or its cover (provided in the kit). Pipette 4 ml of the mixed Detection Buffers on to each membrane and incubate at room temperature for 2 minutes with gentle shaking. Ensure that the detection mixture is evenly covering the membrane without any air bubbles.

16. Gently place the membrane with forceps (antibody side up) on a plastic sheet (provided) and cover the membrane with

another plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane. Work as quickly as possible.

17. The signal can be detected directly from the membrane using a chemiluminescence imaging system or by exposing the array to x-ray film (we recommend using Kodak X-Omat™ AR film) with subsequent development. Expose the membranes for 40 seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce exposure time (eg, 5–30 seconds). If the signals are too weak, increase exposure time (eg, 5–20 min or overnight). Or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

18. Save membranes at –20 °C to –80 °C for future reference.

V. Antibody Array Map

RayBio® L-Series Human Obesity Antibody Array 1 Maps – if needed, larger versions of these maps can obtained by contacting technical support at 770-729-2992 or techsupport@raybiotech.com.

RayBio® L-Series Human Obesity Antibody Array 1 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	α-HRP 1	α-HRP 2	α-HRP 3	Blank	Blank	Blank	Blank	Blank	9	10	11	12	13	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	24	25	26	27	28	29	30
2	α-HRP 1	α-HRP 2	α-HRP 3	Blank	Blank	Blank	Blank	Blank	9	10	11	12	13	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	24	25	26	27	28	29	30
3	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	39	40	41	42	43	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	54	55	56	57	58	59	60
4	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	39	40	41	42	43	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	54	55	56	57	58	59	60
5	61	62	63	64	65	66	67	68	69	70	71	72	73	Blank	Blank	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
6	61	62	63	64	65	66	67	68	69	70	71	72	73	Blank	Blank	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
7	91	92	93	94	95	96	97	98	99	100	101	102	103	Blank	Blank	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
8	91	92	93	94	95	96	97	98	99	100	101	102	103	Blank	Blank	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
9	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	129	130	131	132	133	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	144	145	146	147	148	149	150
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11	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	159	160	161	162	163	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	174	175	176	177	178	179	180
12	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	159	160	161	162	163	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	174	175	176	177	178	179	180
13	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	189	190	191	192	193	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	204	205	206	207	208	209	210
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17	241	242	243	244	245	246	247	248	249	250	251	252	253	Blank	Blank	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
18	241	242	243	244	245	246	247	248	249	250	251	252	253	Blank	Blank	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
19	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	279	280	281	282	283	Blank	Blank	286	287	288	289	290	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
20	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	279	280	281	282	283	Blank	Blank	286	287	288	289	290	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
21	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	α-S 3	α-S 2	α-S 1
22	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	α-S 3	α-S 2	α-S 1
23	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
24	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RayBio® L-Series Human Obesity Antibody Array 1 List

Number	Name	Number	Name	Number	Name
1	P-1a	61	BMP-3	121	Blank
2	P-2a	62	BMP-3b / GDF-10	122	Blank
3	P-3a	63	BMP-4	123	Blank
4	Blank	64	BMP-5	124	Blank
5	Blank	65	BMP-6	125	Blank
6	NEG	66	BMP-7	126	Blank
7	NEG	67	BMP-8	127	Blank
8	Blank	68	BMP-15	128	Blank
9	ACE	69	BMPR-IA / ALK-3	129	IGFBP-1
10	ACE-2	70	BMPR-IB / ALK-6	130	IGFBP-2
11	ACTH	71	BMPR-II	131	IGFBP-3
12	ADFP	72	b-NGF	132	IGF-II
13	Acrp 30	73	C3a des Arg	133	IL-1 R1
14	Blank	74	Blank	134	Blank
15	Blank	75	Blank	135	Blank
16	P-2a	76	CART	136	Blank
17	P-2b	77	CD137 (4-1BB)	137	Blank
18	P-2c	78	CD36	138	Blank
19	Blank	79	Clusterin	139	Blank
20	Blank	80	CNTF	140	Blank
21	NEG	81	C-peptide	141	Blank
22	NEG	82	CRP	142	Blank
23	Blank	83	Cystatin C	143	Blank
24	Adipsin (Factor D)	84	Dtk	144	IL-1 R4
25	AgRP	85	EGF	145	IL-1a
26	AMPKa1	86	EGF-R	146	IL-1b
27	Amylin	87	ENA-78	147	IL-1ra
28	Angiopoietin-1	88	Endophin Beta	148	IL-6
29	Angiopoietin-2	89	Epiregulin	149	IL-6 sR
30	Angiotensinogen / Angiotensin II	90	E-selectin	150	IL-8
31	Blank	91	ET-1 (Endothelin)	151	Blank
32	Blank	92	FABP4	152	Blank
33	Blank	93	FAM3B	153	Blank
34	Blank	94	FAS / Apo-1	154	Blank
35	Blank	95	FGF-10	155	Blank
36	Blank	96	FGF-6	156	NEG
37	Blank	97	FSH	157	NEG
38	Blank	98	Galectin -1	158	Blank
39	Ang-like Factor	99	GH (Growth Hormone)	159	IL-10
40	ANGPTL1	100	Ghrelin	160	IL-11
41	ANGPTL2	101	GITR	161	IL-12
42	ANGPTL3	102	GITRL	162	IL-25 / IL-17E
43	ANGPTL4	103	GLP-1	163	INSL3
44	Blank	104	Blank	164	Blank
45	Blank	105	Blank	165	Blank
46	Blank	106	Glucagon	166	P-3a
47	Blank	107	Glut1	167	P-3b
48	Blank	108	Glut2	168	P-3c
49	Blank	109	Glut3	169	Blank
50	Blank	110	Glut5	170	Blank
51	Blank	111	Glutathione peroxidase 1	171	NEG
52	Blank	112	Glutathione peroxidase 3	172	NEG
53	Blank	113	GROa	173	Blank
54	Apelin Receptor	114	HCC4	174	INSRR
55	ApoB	115	HGF	175	Insulin
56	ApoE	116	HSD-1	176	Insulin R (CD220)
57	Axl	117	ICAM1	177	Leptin
58	BDNF	118	IFNg	178	Leptin R
59	bFGF	119	IGF-1	179	LH (Luteinizing Hormone)
60	BMP-2	120	IGF-1 sR	180	LIF

RayBio® L-Series Human Obesity Antibody Array 1 List …continued

Number	Name	Number	Name	Number	Name
181	Blank	241	PYY	301	Blank
182	Blank	242	RANTES	302	Blank
183	Blank	243	RBP4	303	Blank
184	Blank	244	RELMb	304	Blank
185	Blank	245	Resistin	305	Blank
186	Blank	246	S100	306	NEG
187	Blank	247	S100 A8+A9	307	NEG
188	Blank	248	S100 A10	308	Blank
189	LOX	249	SAA	309	Blank
190	Lymphotactin	250	SDF-1	310	Blank
191	MCP-1	251	SEMA3A	311	Blank
192	MCP-3	252	Serotonin	312	Blank
193	M-CSF	253	Syndecan-3	313	Blank
194	Blank	254	Blank	314	Blank
195	Blank	255	Blank	315	Blank
196	Blank	256	TACE	316	Blank
197	Blank	257	TDAG51	317	Blank
198	Blank	258	TECK	318	Blank
199	Blank	259	TGF-a	319	Blank
200	Blank	260	TGF-b	320	Blank
201	Blank	261	Thrombospondin 1	321	Blank
202	Blank	262	Thrombospondin 2	322	Blank
203	Blank	263	Thrombospondin 4	323	Blank
204	MIF	264	TIMP-1	324	NEG
205	MIP-1a	265	TIMP-2	325	NEG
206	MIP-1b	266	TIMP-3	326	Blank
207	MIP-3b	267	TIMP-4	327	Blank
208	MMP-2	268	Tissue factor (CD142)	328	P-3b
209	MMP-9	269	TLR2	329	P-2b
210	MMP-11	270	TLR4	330	P-1b
211	MMP-19	271	Blank		
212	MSHa	272	Blank		
213	MSPa	273	Blank		
214	Myostatin	274	Blank		
215	NAIP	275	Blank		
216	NeuroD1	276	Blank		
217	Neuropilin-2	277	Blank		
218	NGF R	278	Blank		
219	NPY (Neuropeptide Y)	279	TNF alpha		
220	Obestatin R (GPR-39)	280	TNF sRI		
221	Orexin A	281	TNF sRII		
222	Orexin B	282	TSG-6		
223	OSM	283	TSH		
224	Blank	284	Blank		
225	Blank	285	Blank		
226	Osteocalcin	286	Vaspin		
227	Osteonectin	287	VCAM1		
228	Osteoprotegerin	288	VEGF		
229	PARC	289	Visfatin/PBEF1		
230	PDGF	290	XEDAR		
231	PDGF-AA	291	Blank		
232	PDGF-AB	292	Blank		
233	PDGF-C	293	Blank		
234	PDGF-D	294	Blank		
235	PEDF	295	Blank		
236	Pentraxin-3	296	Blank		
237	PPARg2 / NRIC3	297	Blank		
238	Pref-1	298	Blank		
239	Prohibitin	299	Blank		
240	Prolactin	300	Blank		

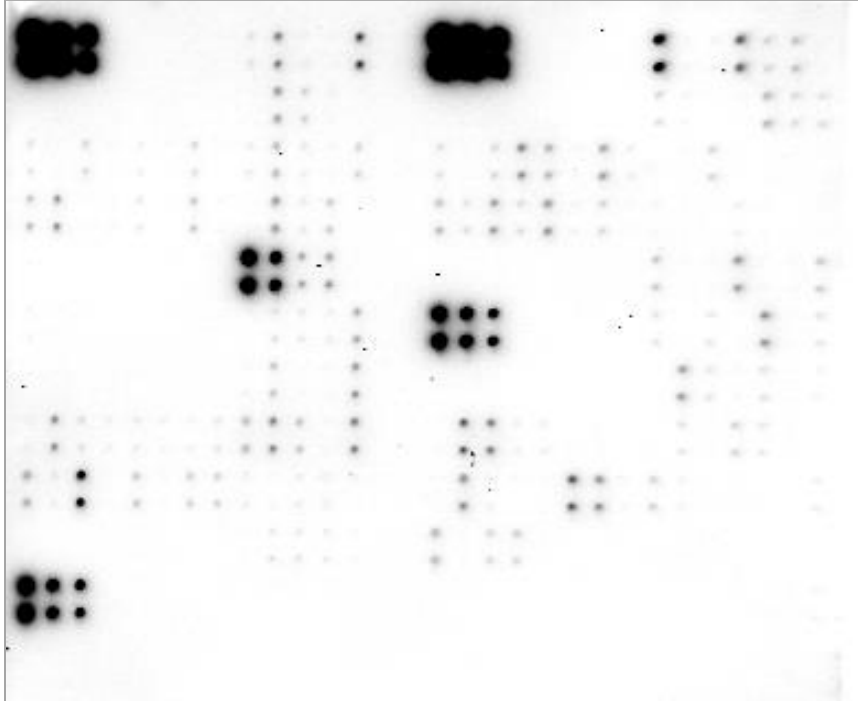
VI. Interpretation of Results

To obtain optimal results, it is suggested to try several different exposure times until the best one is determined. Then, by comparing the signal intensities, relative expression levels of the target proteins can be made. The intensities of signals can be quantified by densitometry. Anti-HRP (P-1a, P-2a, P-3a) and anti-streptavidin (P-1b, P-2b, P-3b) will produce positive control signals, which can be used to identify the orientation and help normalize the results from different arrays being compared.

Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies.

The RayBio® Analysis Tool is a program specifically designed for analysis of RayBio® L-Series Rat Antibody Antibody Arrays. This tool will not only assist in compiling and organizing your data, but also reduces your calculations to a "copy and paste." Call RayBiotech, Inc. at 770-729-2992 for ordering information.

L-Series Human Obesity Array 1 image



VII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak signal or no signal	1. Taking too much time for detection.	1. The whole detection process must be completed in 30 min.
	2. Film developer does not work properly.	2. Fix film developer.
	3. Did not mix HRP-streptavidin well before use.	3. Mix tube containing HRP-Conjugate Streptavidin well before use since precipitates may form during storage.
	4. Sample is too dilute.	4. Increase sample concentration
	5. Other.	1.Check if there were any contamination with any solution containing amines in biotin-labeling step
		2. Slightly increase HRP concentrations.
		3. Work as quickly as possible after mix Detection Buffer C and D
		4. Expose film for overnight to detect weak signal.
Uneven signal	1. Bubbles formed during incubation.	1. Remove bubbles during incubation.
	2. Membranes were not completely covered by solution.	2. Completely cover membranes with solution.
High background	1. Exposure time is too long.	1. Decrease exposure time.
	2. Membranes dry out during experiment.	2. Completely cover membranes with solution during experiment. Cover tray w/ lid
	3. Sample is too concentrated.	3. Dilute sample.

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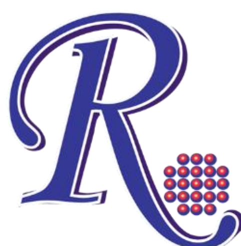

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