RayBio® Label-Based (L-Series) Human Antibody Array L-1000 Membrane Kit

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

Patent Pending Technology
User Manual (January 1, 2022)

For the simultaneous detection of the relative expression of 1000 human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates and other body fluids.

L-Series Human Antibody Array L-1000
Cat# AAH-BLM-1000-2 (2 Sample Kit)
Cat# AAH-BLM-1000-4 (4 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

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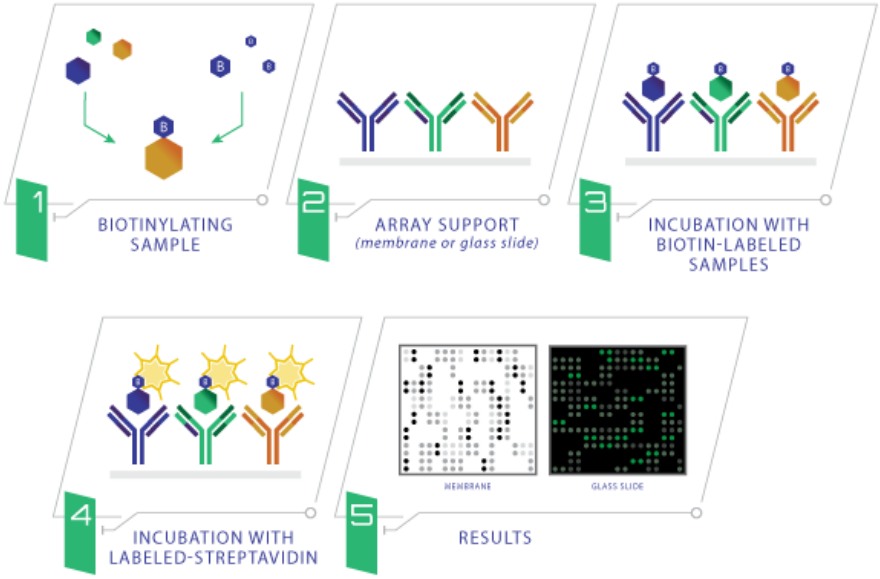
I. Introduction

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 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, the kit should be stored at 4 °C or below and must be used within 6 months from the date of shipment. For longer period of storage, Labeling Reagent (Item B) and Array Membrane (Item E) should be stored at -20 °C and avoid repeated freeze-thaw cycles. Labeling Reagent (Item B) should be prepared fresh before use. After initial use, Labeling Buffer, Blocking Buffer, Stop Solution, HRP-Conjugated Streptavidin, and 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).

ITEM	DESCRIPTION	2 SAMPLE KIT	4 SAMPLE KIT
B	Labeling Reagent	2 vials	4 vials
C	Labeling Buffer	1 bottle (30 ml)	1 bottle (30 ml)
D	Stop Solution	1 vial (50 µl)	1 vial (50 µl)
E	L-series Antibody Array Membranes	2 membranes each of Human L-507 and L-493	4 membranes each of Human L-507 and L-493
F	4X Blocking Buffer	1 bottle (30 ml)	2 bottles (30 ml)
I	500X HRP-Conjugated Streptavidin Concentrate	1 vial (100 µl)	2 vials (100 µl)
K	Detection Buffer C	1 bottle (10 ml)	2 bottles (10 ml)
L	Detection Buffer D	1 bottle (10 ml)	2 bottles (10 ml)
G	20X Wash Buffer 1 Concentrate	1 bottle (30 ml)	2 bottles (30 ml)
H	20X Wash Buffer 2 Concentrate	1 bottle (30 ml)	2 bottles (30 ml)
J-2	Spin Columns (10 ml)	4 columns	8 columns
N/A	Plastic Incubation Trays (w/lid)	4 trays	8 trays
N/A	2X Lysis Buffer	1 bottle (10 ml)	1 bottle (10 ml)
Other Kit Components: Plastic Sheets			

B. Additional Materials Required

- 2-5 ml tube, small plastic or glass containers
- 50 ml conical collection tubes
- Orbital shaker or oscillating rocker
- Kodak X-Omat™ AR film (REF 165 1454) and film processor
  or Chemiluminescence imaging system

- Pipettors, pipette tips and other common lab consumables
- Eppendorf tube

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.**,†
4. To collect supernatants, centrifuge at 1,000 x g for 10 min 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 densitometry 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 the 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 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. Determine 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 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 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.

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 Array Membranes

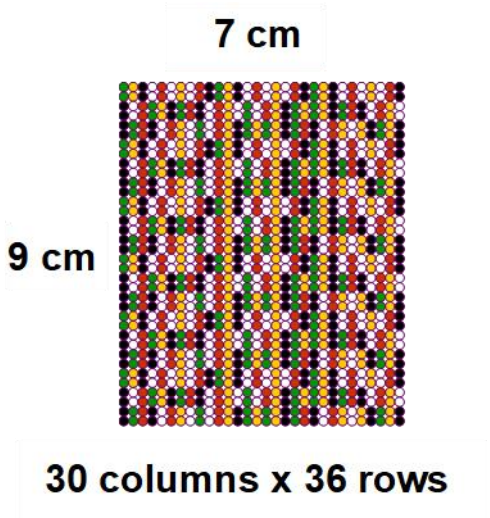
- Always use forceps to handle membranes and grip the membranes by the edges only.
- Never allow membranes to dry during the experiment.
- Avoid touching membranes with hands or any sharp tools.

C. Incubations of Antibody Array

- Completely cover membranes with sample or buffer during incubation and cover the Plastic Incubation Tray with the 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 (sample incubation) or step 7 (HRP-Conjugated Streptavidin incubation) may be done at 4 °C overnight.

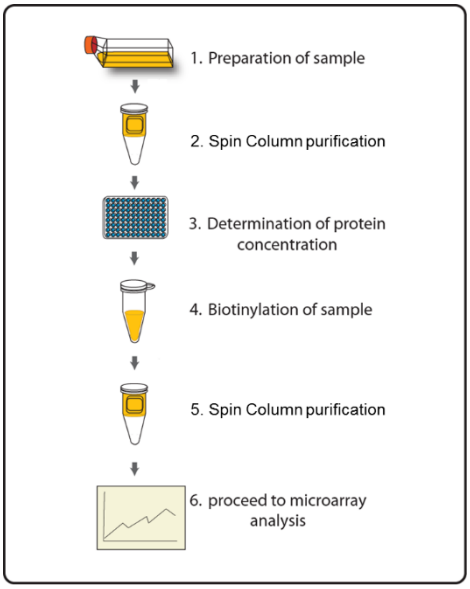
D. Layout of Array Membrane



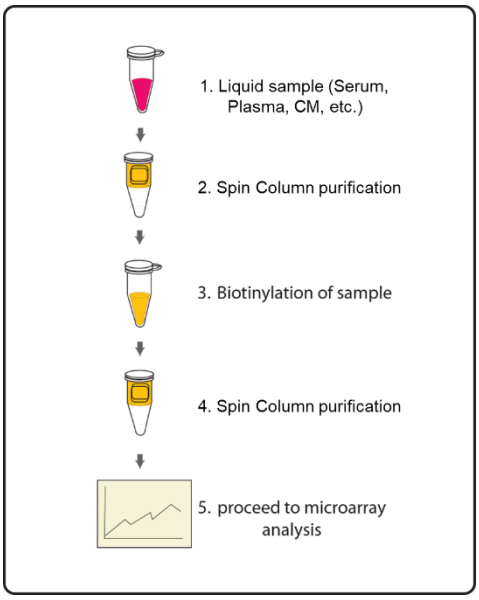
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



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



A. Sample purification

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

1. Twist to remove the bottom closure of the Spin column and loosen the cap (Do not remove).

2. Place the Spin column into a 50 ml conical collection tube, centrifuge at 1,000 x g for 3 minutes to remove the storage buffer. Discard the flow-through.

3. Wash the column three times with 5 ml ultrapure water (ddH2O) or 1xPBS (pH8.0) each, centrifuge 1,000 x g for 3 minutes to remove the

flow-through. Blot the bottom of the column to remove excess liquid, and transfer device to a new collection tube.

4. Apply sample on top of the resin within the next few minutes. Centrifuge at 1,000 x g for 3 minutes to collect the flow-through that contains sample. The recommended sample dilution as following:

    - *Culture Media (CM): 2 ml neat supernatant*
    - *Serum/Plasma: 25 µl serum/plasma in 2 ml labeling buffer*
    - *Cell/tissue lysate: 100 µg lysate in 1 ml labeling buffer*

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

Note: The maximal sample volume is 4 ml for each Spin Column. Do not load over 4 ml 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 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 40 µl of Labeling Reagent into the sample tube (for 2 ml supernatant).

    b. For labeling serum or plasma: Add 40 µl of Labeling Reagent Solution into the sample tube (for 25 µl serum/plasma *in 2 ml labeling buffer*).

c. For labeling cell or tissue lysates: Add 8 µl of Labeling Reagent Solution into the sample tube (for 100 µg lysate *in 1 ml 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 more or less amount sample is labelled, adjust this volume proportionally.

7. Add 5 µl Stop Solution (Item D) into each reaction 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. Blocking and Incubations

8. 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.

   Note: Up to 4 membranes can be incubated together within one tray with proportional amount of reaction buffer. Rotate the membrane sequence at least once during sample incubation if more than one membrane is incubated in one tray.

9. Dilute 4X Blocking Buffer (Item F) with deionized or distilled water to prepare the 1X Blocking Buffer. Add 6 ml of 1X Blocking Buffer to each

membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour.

10. Aspirate the Blocking Buffer from each tray. Add 6 ml of diluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours.

Note: It is recommended to use 10-20 folds diluted biotin-labeled culture supernatant, 10-20 folds diluted biotin-labeled serum/plasma, 100 folds diluted biotin-labeled cell/tissue lysate, or 10-20 folds for other body fluids. Dilute sample using 1X Blocking Buffer. The optimal concentration of sample used will depend on the abundance of target 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: Incubation may be done at room temperature with gentle shaking for 2 hours or overnight at 4°C.

11. Dilute 20X Wash Buffer 1 (Item G) 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.

12. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 (Item H) 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.

13. Aspirate the 1X Wash Buffer 2 from each tray.

14. Prepare the HRP-Conjugated Streptavidin. Briefly spin down the tube containing the 500X HRP-Conjugated Streptavidin (Item I)

immediately before use. Dilute the 500X HRP-Conjugated Streptavidin with 1X Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. Pipette up and down to mix gently. Add 6 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.

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

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

16. Wash as directed in steps 11 through 13.

D. Detection

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

17. 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 (There is a "-" symbol on 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 onto 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.

18. 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.

19. 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 the exposure time (e.g., 5–30 seconds). If the signals are too weak, increase the exposure time (e.g., 5–20 min or overnight) or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

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

V. Antibody Array Maps

A. RayBio® Human Antibody Array L-507 Array Map

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B. RayBio® Human Antibody Array L-493 Array Map

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17	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
18	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263
19	Blank	Blank	Blank	Blank	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289
20	Blank	Blank	Blank	Blank	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289
21	Blank	Blank	Blank	Blank	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
22	Blank	Blank	Blank	Blank	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
23	Blank	Blank	Blank	Blank	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341
24	Blank	Blank	Blank	Blank	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341
25	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371
26	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371
27	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401
28	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401
29	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431
30	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431
31	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461
32	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461
33	Blank	Blank	Blank	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	Blank	Blank	Blank
34	Blank	Blank	Blank	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	Blank	Blank	Blank
35	Blank	Blank	Blank	Blank	486	487	488	489	490	491	492	493	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS3	POS2	POS1
36	Blank	Blank	Blank	Blank	486	487	488	489	490	491	492	493	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	POS3	POS2	POS1

VI. Antibody Array Target Lists

A. RayBio® Human Antibody Array L-507 Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	6Ckine	74	CNTF R alpha	147	FGF-19	220	IGFBP-4	293	IL-22 BP	366	MMP-24	439	Shh-N
2	Activin A	75	F3	148	FGF-20	221	IGFBP-6	294	IL-22 R	367	MMP-25	440	SPARC
3	Activin B	76	CRIM 1	149	FGF-21	222	IGFBP-rp1	295	IL-23	368	Musk	441	Spinesin
4	Activin C	77	Cripto-1	150	FGF-23	223	IGF-I	296	IL-23 R	369	MSPa	442	TACI
5	Activin RIA	78	CRTH-2	151	FLRG	224	IGF-I R	297	IL-24	370	MICA	443	Tarc
6	Activin RIB	79	Cryptic	152	Flt-3 Ligand	225	IGF-II	298	IL-26	371	NAP-2	444	TCCR
7	EYA2	80	CTACK	153	Follistatin	226	IGF-II R	299	IL-27	372	NCAM-1	445	TECK
8	Activin RIIA	81	CTGF	154	Follistatin-like 1	227	IL-1 alpha	300	IL-28A	373	Neuritin	446	TFPI
9	Adiponectin	82	CTLA-4	155	Fractalkine	228	IL-1 beta	301	IL-29	374	NeuroD1	447	TGF-alpha
10	AgRP	83	CV-2	156	Frizzled-1	229	IL-1 F5	302	IL-31	375	Neuropilin-2	448	TGF-beta 1
11	ALCAM	84	CXCL14	157	Frizzled-3	230	IL-1 F6	303	IL-31 RA	376	Neurturin	449	TGF-beta 2
12	Angiogenin	85	CXCL16	158	Frizzled-4	231	IL-1 F7	304	BACE-1	377	NGF R	450	TGF-beta 3
13	Angiopoietin-1	86	CXCR1	159	Frizzled-5	232	IL-1 F8	305	FACX	378	NOV	451	ATP2B1
14	Angiopoietin-2	87	CXCR2	160	Frizzled-6	233	IL-1 F9	306	Insulin	379	GGF2	452	TGF-beta RI
15	Angiopoietin-4	88	CXCR3	161	Frizzled-7	234	IL-1 F10	307	Insulin R	380	Nidogen-1	453	TGF-beta RII
16	ANGPTL1	89	CXCR4	162	Galectin-3	235	IL-1 R3	308	Insulysin	381	NrCam	454	Grb2
17	ANGPTL2	90	CXCR5	163	GASP-1	236	IL-1 R4	309	IP-10	382	NRG2	455	TGF-beta RIII
18	ANGPTL7	91	CXCR6	164	GASP-2	237	IL-1 R6	310	I-TAC	383	NRG3	456	Thrombopoietin
19	Angiostatin	92	D6	165	GCP-2	238	IL-1 R8	311	Kininostatin	384	NT-3	457	Thyroid Peroxidase
20	APJ	93	DAN	166	GCSF	239	IL-1 R9	312	Kremen-1	385	NT-4	458	Thrombospondin-1
21	Amphiregulin	94	DANCE	167	G-CSF R	240	IL-1 ra	313	Kremen-2	386	Orexin A	459	Thrombospondin-2
22	APRIL	95	DcR3	168	GDF1	241	IL-1 RI	314	Lck	387	Orexin B	460	Thrombospondin-4
23	Artemin	96	Decorin	169	GDF3	242	IL-1 RII	315	LTBP1	388	OSM	461	Thymopoietin
24	Axl	97	Dkk-1	170	GDF5	243	IL-2	316	LBP	389	Osteoactivin	462	Tie-1
25	B7-1	98	Dkk-3	171	GDF8	244	IL-2 R alpha	317	LECT2	390	Osteocrin	463	Tie-2
26	BAFF R	99	Dkk-4	172	GDF9	245	IL-2 R beta	318	Lefty-A	391	Osteoprotegerin	464	TIMP-1
27	BCMA	100	DR3	173	GDF11	246	IL-2 R gamma	319	Leptin R	392	OX40 Ligand	465	TIMP-2
28	BD-1	101	DR6	174	GDF-15	247	IL-3	320	Leptin	393	PARC	466	TIMP-3
29	BDNF	102	Dtk	175	GDNF	248	IL-3 R alpha	321	LFA-1 alpha	394	PD-ECGF	467	TIMP-4
30	beta-Catenin	103	EDA-A2	176	GFR alpha-1	249	IL-4	322	LIF	395	PDGF R alpha	468	DEFA5
31	Bax	104	EDAR	177	GFR alpha-2	250	IL-4 R	323	LIF R alpha	396	PDGF R beta	469	TLR1
32	beta-NGF	105	EDG-1	178	GFR alpha-3	251	IL-5	324	LIGHT	397	PDGF-AA	470	TLR2
33	BIK	106	EGF	179	GFR alpha-4	252	IL-5 R alpha	325	Lipocalin-1	398	PDGF-AB	471	TLR3
34	BLC	107	EGF R	180	GITR	253	IL-6	326	LRP-1	399	PDGF-BB	472	TLR4
35	BMP-2	108	EG-VEGF	181	GITR Ligand	254	IL-6 R	327	LRP-6	400	PDGF-C	473	TMEFF1
36	BMP-3	109	EMAP-II	182	CBR1	255	IL-7	328	L-Selectin	401	PDGF-D	474	TMEFF2
37	BMP-3b	110	ENA-78	183	Glut1	256	IL-7 R alpha	329	Lipocalin-2	402	PECAM-1	475	TNF-alpha
38	BMP-4	111	Endocan	184	Glut2	257	IL-8	330	Lymphotactin	403	Pentraxin3	476	TNF-beta
39	BMP-5	112	Endoglin	185	Glut3	258	IL-9	331	LTB	404	Persephin	477	TNF RI
40	BMP-6	113	Endostatin	186	Glut5	259	IL-10	332	LTBR	405	PF4	478	TNF RII
41	BMP-7	114	EN-RAGE	187	Glypican 3	260	IL-10 R alpha	333	MAC-1	406	PlGF	479	TRADD
42	BMP-8	115	Eotaxin	188	Glypican 5	261	IL-10 R beta	334	MCP-1	407	PLUNC	480	TRAIL
43	BMP-15	116	Eotaxin-2	189	GM-CSF	262	IL-11	335	MCP-2	408	Pref-1	481	TRAIL R1
44	BMPR-IA	117	Eotaxin-3	190	GM-CSF R alpha	263	IL-12 p40	336	MCP-3	409	Progranulin	482	TRAIL R2
45	BMPR-IB	118	Epiregulin	191	Granzyme A	264	IL-12 p70	337	MCP-4	410	Prolactin	483	TRAIL R3
46	BMPR-II	119	ErbB2	192	GREMLIN	265	IL-12 R beta 1	338	M-CSF	411	P-selectin	484	TRAIL R4
47	BTC	120	ErbB3	193	GRO	266	IL-12 R beta 2	339	M-CSF R	412	RAGE	485	TRANCE
48	Cardiotrophin-1	121	ErbB4	194	GRO-a	267	IL-13	340	MDC	413	RANK	486	TREM-1
49	CCL14	122	Erythropoietin	195	GH	268	IL-13 R alpha 1	341	MFG-E8	414	RANTES	487	TROY
50	CCL28	123	E-Selectin	196	GHR	269	IL-13 R alpha 2	342	MFRP	415	RELM beta	488	TSG-6
51	CCR1	124	Endothelin	197	HB-EGF	270	IL-15	343	MIF	416	RELT	489	TSLP R
52	CCR2	125	FADD	198	HCC-4	271	IL-15 R alpha	344	MIG	417	ROBO4	490	TWEAK
53	CCR3	126	FAM3B	199	HCR	272	IL-16	345	MIP-1a	418	S100 A8/A9	491	TWEAK R
54	CCR4	127	Fas	200	Hepassocin	273	IL-17	346	MIP-1b	419	S100A10	492	Ubiquitin+1
55	CCR5	128	Fas Ligand	201	GLO-1	274	IL-17B	347	MIP-1d	420	SAA	493	uPA
56	CCR6	129	FGF Basic	202	HGF	275	IL-17B R	348	MIP 2	421	SCF	494	uPAR
57	CCR7	130	FGF-BP	203	HGFR	276	IL-17C	349	MIP-3 alpha	422	SCF R	495	Vasorin
58	CCR8	131	FGF R3	204	HRG-alpha	277	IL-17D	350	MIP-3 beta	423	SDF-1	496	VCAM-1
59	CCR9	132	FGF R4	205	HRG-beta 1	278	IL-17E	351	MMP-1	424	sFRP-1	497	VE-Cadherin
60	CD14	133	FGF R5	206	HVEM	279	IL-17F	352	MMP-2	425	sFRP-3	498	VEGF
61	CD27	134	FGF-4	207	I-309	280	IL-17R	353	MMP-3	426	sFRP-4	499	VEGF R2
62	CD30	135	FGF-5	208	ICAM-1	281	IL-17RC	354	MMP-7	427	sgp130	500	VEGF R3
63	CD30 Ligand	136	FGF-6	209	ICAM-2	282	IL-17RD	355	MMP-8	428	SIGIRR	501	VEGF-B
64	CD40	137	FGF-7	210	ICAM-3	283	IL-18 BPa	356	MMP-9	429	Siglec-5	502	VEGF-C
65	CD40 Ligand	138	FGF-8	211	ICAM-5	284	IL-18 R alpha	357	MMP-10	430	Siglec-9	503	VEGF-D
66	CD 163	139	FGF-9	212	IFN-alpha/beta R1	285	IL-18 R beta	358	MMP-11	431	SLPI	504	VEGI
67	Cerberus 1	140	FGF-10	213	IFN-alpha/beta R2	286	IL-19	359	MMP-12	432	Smad 1	505	WIF-1
68	Chem R23	141	FGF-11	214	IFN-beta	287	IL-20	360	MMP-13	433	Smad 4	506	WISP-1
69	Chordin-Like 1	142	FGF-12	215	IFN-gamma	288	IL-20 R alpha	361	MMP-14	434	Smad 5	507	XEDAR
70	Chordin-Like 2	143	FGF-13 1B	216	IFN-gamma R1	289	IL-20 R beta	362	MMP-15	435	Smad 7		
71	Csk	144	FGF-16	217	IGFBP-1	290	IL-21	363	MMP-16	436	Smad 8		
72	CLC	145	FGF-17	218	IGFBP-2	291	IL-21 R	364	MMP-19	437	Prdx6		
73	CNTF	146	FGF-18	219	IGFBP-3	292	IL-22	365	MMP-20	438	Soggy-1		

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

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	11b-HSD1	73	BMX	145	CRTAM	217	FRK	289	KLF4	361	PI 16	433	SCGF
2	2B4	74	BNIP2	146	CSH1	218	ARB1	290	LAG-3	362	PIK3R1	434	SOST
3	4-1BB	75	BNP	147	Troponin T	219	Furin	291	Layilin	363	PIM2	435	SOX17
4	A1BG	76	Btk	148	CutA	220	Fyn	292	LDL R	364	PKM2	436	SOX2
5	A2M	77	C2	149	Cyclin D1	221	GADD45A	293	Legumain	365	Plasminogen	437	SPARCL1
6	ABL1	78	C3a	150	Cystatin A	222	Galanin	294	LH	366	Podocalyxin	438	SPINK1
7	ACE	79	C5a	151	Cystatin B	223	Galectin-1	295	LIMPII	367	POMC	439	SRMS
8	ACE-2	80	C7	152	Cystatin C	224	Galectin-3BP	296	LIN41	368	PON1	440	SSEA-1
9	ACK1	81	C8b	153	Cytochrome C	225	Galectin-7	297	Livin	369	PON2	441	SSEA-4
10	ACPP	82	C9	154	Cytokeratin 8	226	gamma-Thrombin	298	LOX-1	370	PPARg2	442	SSTR2
11	ACTH	83	CA9	155	Cytokeratin 18	227	Gas1	299	LPS	371	PPP2R5C	443	SSTR5
12	ADAM-9	84	CA15-3	156	Cytokeratin 19	228	Gastrin	300	LRG1	372	Presenilin 1	444	Survivin
13	ADAMTS1	85	CA19-9	157	DBI	229	GATA-3	301	LTF	373	Presenilin 2	445	SYK
14	ADAMTS10	86	CA125	158	DCBLD2	230	GATA-4	302	LTK	374	Pro-BDNF	446	Syndecan-1
15	ADAMTS13	87	Cadherin-13	159	D-Dimer	231	Gelsolin	303	Lumican	375	Procalcitonin	447	Syndecan-3
16	ADAMTS15	88	CLEC14A	160	DEFA1/3	232	Ghrelin	304	Lyn	376	Pro-Cathepsin B	448	TACE
17	ADAMTS17	89	Calbindin D	161	CPA1	233	GLP-1	305	LYRIC	377	Progesterone	449	TAF4
18	ADAMTS18	90	Calcitonin	162	Desmin	234	GMNN	306	LYVE-1	378	pro-Glucagon	450	Tec
19	ADAMTS19	91	Calreticulin	163	DLL1	235	GPBB	307	LZTS1	379	Prohibitin	451	TFF1
20	ADAMTS4	92	Calsyntenin-1	164	DLL4	236	GPI	308	Mammaglobin A	380	Pro-MMP-7	452	TFF3
21	ADAMTS5	93	CART	165	DMP-1	237	GPR-39	309	Marapsin	381	Pro-MMP-9	453	Thrombin
22	ADAMTSL2	94	Caspase-3	166	DPPIV	238	GPX1	310	MATK	382	Pro-MMP-13	454	Thrombomodulin
23	Adipsin	95	Caspase-8	167	E-Cadherin	239	GPX3	311	MBL	383	ProSAAS	455	TK1
24	Afamin	96	Cathepsin B	168	Endorphin Beta	240	GRP	312	C1qTNF1	384	Prostasin	456	Thyroglobulin
25	AFP	97	Cathepsin D	169	EDNRA	241	GRP75	313	Mer	385	Protein p65	457	TIM-1
26	ALBUMIN	98	Cathepsin L	170	Enolase 2	242	GRP78	314	Mesothelin	386	PSA-Free	458	TNK1
27	Aldolase A	99	Cathepsin S	171	ENPP2	243	GSR	315	MICB	387	PSA-total	459	TOPORS
28	Aldolase B	100	CBP	172	EpCAM	244	GST	316	Midkine	388	PSP	460	TPA
29	Aldolase C	101	CCK	173	EphA1	245	HADHA	317	MINA	389	PTH	461	TPM1
30	ALK	102	CD23	174	EphA2	246	HAI-1	318	MSHa	390	PTHLP	462	TRA-1-60
31	Alpha 1 AG	103	CD24	175	EphA3	247	HAI-2	319	MTUS1	391	PTN	463	TRA-1-81
32	A1M	104	CD36	176	EphA4	248	Haptoglobin	320	Myoglobin	392	PTPRD	464	Transferrin
33	Alpha Lactalbumin	105	CD38	177	EphA5	249	hCG alpha	321	NAIP	393	PYK2	465	Trappin-2
34	ALPP	106	CD44	178	EphA6	250	hCGb	322	Nanog	394	PYY	466	TRKB
35	AMICA	107	CD45	179	EphA7	251	Hck	323	NELL2	395	Ras	467	Troponin I
36	AMPKa1	108	CD46	180	EphA8	252	HE4	324	Neprilysin	396	RBP4	468	Troponin C
37	Amylin	109	CD47	181	EphB1	253	Hemopexin	325	Nesfatin	397	RECK	469	TRPC1
38	ANGPTL3	110	CD55	182	EphB2	254	Hepcidin	326	Nestin	398	RELM alpha	470	TRPC6
39	ANGPTL4	111	CD59	183	EphB3	255	HOXA10	327	NET1	399	Resistin	471	TRPM7
40	Annexin A7	112	CD61	184	EphB4	256	HSP10	328	Netrin G2	400	RET	472	Trypsin 1
41	APC	113	CD71	185	EphB6	257	HSP20	329	Netrin-4	401	RIP1	473	TSH
42	APCS	114	CD74	186	ERRa	258	HSP27	330	Neurokinin A	402	ROCK1	474	TSLP
43	Apelin	115	CD79 alpha	187	Erythropoietin R	259	HSP32	331	Neuropeptide Y	403	ROCK2	475	TXK
44	Apex1	116	CD90	188	ESAM	260	HSP40	332	NF1	404	ROR1	476	Tyk2
45	APN	117	CD97	189	EV15L	261	HSP60	333	NM23-H1/H2	405	ROR2	477	TYRO10
46	ApoA1	118	CD200	190	EXTL2	262	HSP70	334	Notch-1	406	ROS	478	Uromodulin
47	ApoA2	119	CEA	191	FABP1	263	HSP90	335	NPTX1	407	RYK	479	Vasopressin
48	ApoA4	120	CEACAM-1	192	FABP2	264	HSPA8	336	NPTXR	408	S100A4	480	VDUP-1
49	ApoB	121	Ceruloplasmin	193	FABP3	265	HTRA2	337	NR3C3	409	S100A6	481	VEGF R1
50	ApoB100	122	CFHR2	194	FABP4	266	IBSP	338	Ntn1	410	S100A8	482	VGF
51	ApoC1	123	Chemerin	195	Fc gamma RIIIB	267	IGF2BP1	339	OCT3/4	411	S-100b	483	VIPR2
52	ApoC2	124	CHI3L1	196	Factor XIII B	268	IGFBP-5	340	Omentin	412	SART1	484	Visfatin
53	ApoC3	125	Chromogranin A	197	FAK	269	IDUA	341	Osteocalcin	413	SART3	485	VDR
54	ApoD	126	Chymase	198	FAP	270	IL-33	342	Osteopontin	414	SCG3	486	VDB
55	ApoE	127	cIAP-2	199	Fcg RIIB/C	271	IL-34	343	OX40	415	Selenoprotein P	487	PROS1
56	ApoE3	128	Ck beta 8-1	200	Fen-1	272	IL-28B	344	p21	416	SEMA3A	488	Vitronectin
57	ApoH	129	CKMB	201	FER	273	INSL3	345	p27	417	Serotonin	489	VWF
58	ApoM	130	Claudin-3	202	Ferritin	274	INSRR	346	p53	418	Serpin G1	490	WT1
59	APP	131	Claudin-4	203	Fetuin A	275	ITGAV	347	PAI-1	419	Serpin A1	491	XIAP
60	ASPH	132	CLEC3B	204	Fetuin B	276	Itk	348	PAK7	420	Serpin A3	492	ZAG
61	Attractin	133	Clusterin	205	FGFR1	277	ITM2B	349	Pancreastatin	421	Serpin A4	493	ZAP70
62	B3GNT1	134	CNDP1	206	FGFR1 alpha	278	Kallikrein 2	350	PP	422	Serpin A5		
63	BAF57	135	COCO	207	FGFR2	279	Kallikrein 5	351	Pappalysin-1	423	Serpin A8		
64	BAFF	136	CFH	208	Fibrinogen	280	Kallikrein 6	352	PARK7	424	Serpin A9		
65	BAI-1	137	Contactin-1	209	Fibrinopeptide A	281	Kallikrein 7	353	P-Cadherin	425	Serpin A12		
66	BCAM	138	Contactin-2	210	Fibronectin	282	Kallikrein 8	354	PCAF	426	Serpin B5		
67	B2M	139	CBG	211	Ficolin-3	283	Kallikrein 10	355	PD-1	427	Serpin D1		
68	Beta Defensin 4	140	COX-2	212	FIH	284	Kallikrein 11	356	PDX-1	428	Serpin I1		
69	Beta IG-H3	141	C-peptide	213	FOLR1	285	Kallikrein 14	357	PEDF	429	SERTAD2		
70	Biglycan	142	CPN2	214	FOXN3	286	KCC3	358	PEPSINOGEN I	430	SHBG		
71	BLAME	143	Creatinine	215	FoxO1	287	KCTD10	359	PEPSINOGEN II	431	SMAC		
72	BMP-9	144	CRP	216	FoxP3	288	KIF3B	360	PGRP-S	432	SNCG		

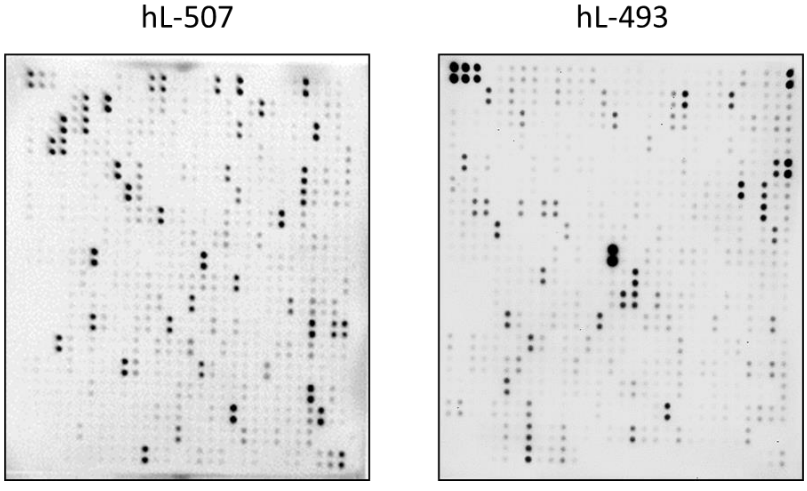
VII. Interpretation of Results:

A. Explanation of Controls Spots

To obtain optimal results using a chemiluminescence imaging system (UVP BioImaging Systems), 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. There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized anti-HRP antibodies, which will produce positive control signals after incubation with HRP-conjugated Streptavidin. With all other variables being equal, the Positive Control intensities will be the same for each sub-array, which allows for inter-array normalization. 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. 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 arrays probed with human serum sample.



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

C. Background Subtraction

Once you have obtained densitometry data, it is recommended to subtract the local background and normalize to the Positive Control signals before proceeding to analysis.

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.

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

Human antibody array L-1000 detects 1000 unique human 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	Taking too much time for detection	The whole detection process must be completed within 30 min.
	Film developer does not work properly.	Fix film developer.
	Did not mix HRP-Streptavidin well before use.	Mix tube containing HRP-Conjugated Streptavidin well before use since precipitates may form during storage.
	Sample is too diluted	Increase sample concentration
	Labeling reagent does not function well	Labeling reagent needs to be saved in -20C and avoid free thaw cycle. Always use fresh labeling reagent for sample labelling.
	Other	Check if there were any contamination with any solution containing amines in biotin-labeling step.
		Slightly increase HRP concentrations.
		Work as quickly as possible after mix Detection Buffer C and D.
Uneven signal	Bubble formed during incubation	Remove bubbles during incubation.
	Membranes were not completely covered with solution	Completely cover membranes with solution.
	Insufficient wash	Use more stringent wash.
High background	Exposure time is too long	Decrease exposure time.
	Membranes dry out during experiment.	Completely cover membranes with solution during experiment. Cover tray with lid.
	Sample is too concentrated.	Dilute sample.

IX. Selected References

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