RayBio® Label-Based (L-Series) Mouse L2 Array, Glass Slide

Patent Pending Technology
User Manual (Jan 1, 2022)

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.

AAM-BLG-2-4 (4 Sample Kit)
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

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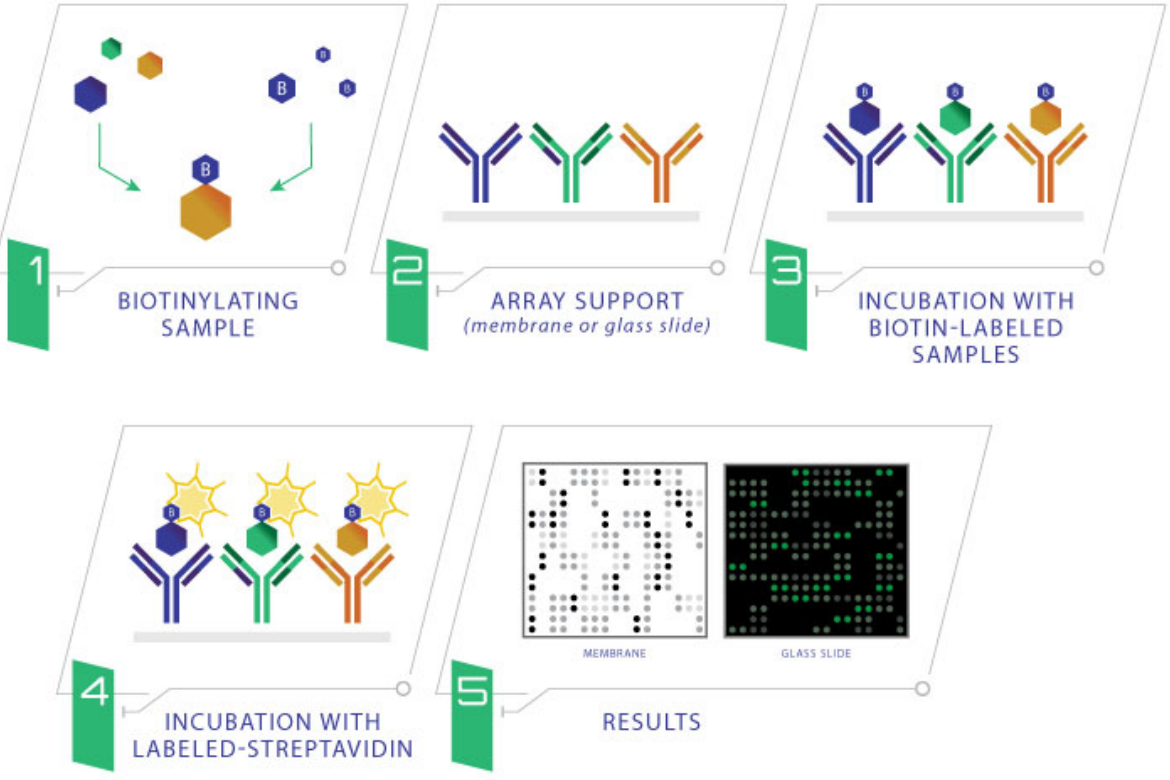
I. Introduction

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 glass slide arrays are then blocked, just like a western blot, and the biotin-labeled sample is added onto the glass slide, which is pre-printed with capture antibodies. The slide is incubated to allow binding of target proteins. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then applied to the array. Finally, the glass slide is dried, and laser fluorescence scanning is used to visualize the signals.



II. Materials Provided

A. Storage Recommendations

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

ITEM	DESCRIPTION	4 SAMPLE KIT	8 SAMPLE KIT
A	Spin Columns (0.5ml)	8 columns	16 columns
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	1 vial (50 µl)
E	RayBio® L-Series Glass Slide*	1 slide	2 slides
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 equivalent-Conjugated Streptavidin	1 vial	2 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (30 ml)	1 bottle (30 ml)
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	1 bottle (10 ml)
M	30 ml Centrifuge Tube	1 tube	1 tube

*Each slide contains 4 identical subarrays
**Only needed if testing cell or tissue lysates

B. Additional Materials Required

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

III. Overview and General Considerations

A. Preparation and Storage of Samples

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

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

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

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

2. Extracting Protein from Cells
    1. Centrifuging Cells
        a. Adherent Cells:
            i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.
            ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.
        b. Cells in Suspension: Pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 minutes.
    2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.
    3. Pipette up and down to resuspend cells and rock the lysates gently at 2-8°C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8°C.

        Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.
    4. Transfer lysates to a clean tube. Determining cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.
3. Extracting Protein from Crude Tissue
    1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O).
    2. Homogenize the tissue according to homogenizer manufacturer instructions.

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

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

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

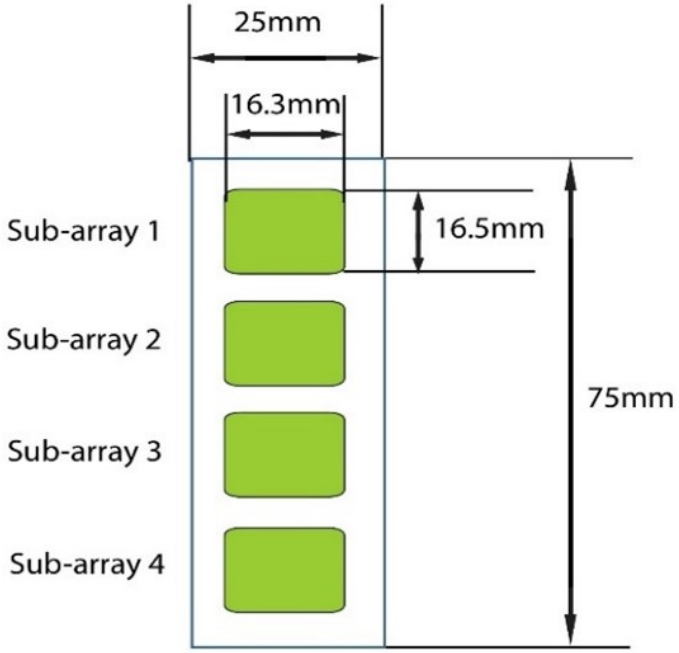
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

B. Handling the Glass Slides

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

C. Layout of Array Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

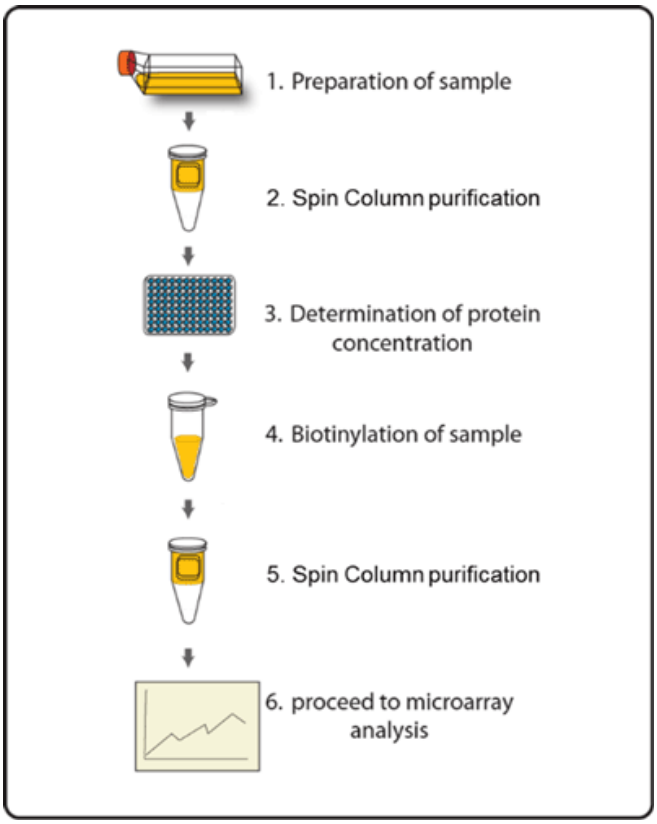
D. Incubations and Washes

- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 4°C
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.
- Unlike most Cy3 fluors, the streptavidin-conjugated fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from direct, strong light and temperatures above RT.

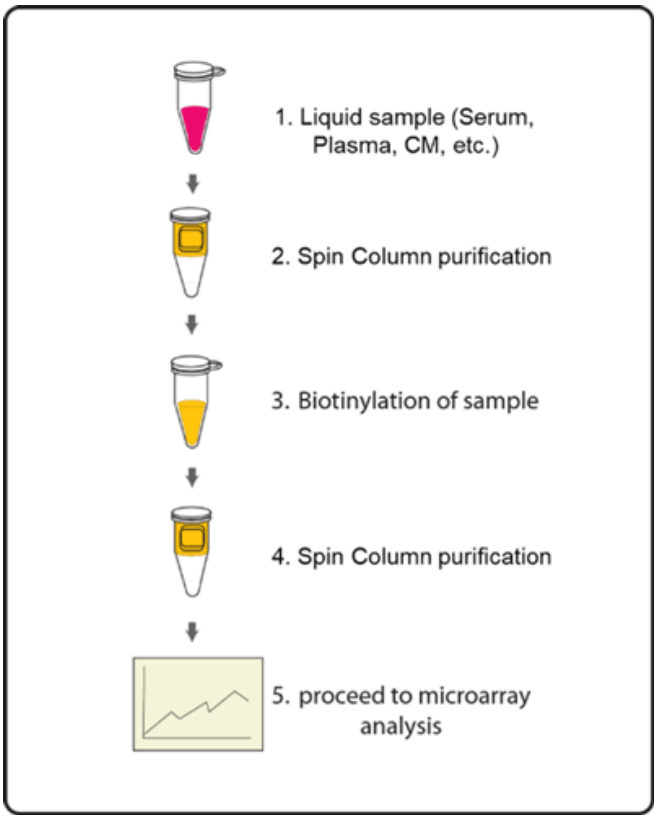
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



2. Serum, plasma, or Cell culture supernatants



A. Sample purification

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

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).
2. Place the Spin Column into a collection tube and centrifuge at 1,500 x g for 1 minute to remove the storage buffer. Discard the flow-through.
3. Wash the Spin Column three times with 300 µl Labeling Buffer each, centrifuge at 1,500 x g for 1 minute to remove the flow-through. Discard the flow-through and blot the bottom of the column to remove excess liquid. Transfer the Spin Column to a new collection tube.

4. Apply sample on top of the resin within the next few minutes. Centrifuge at 1,500 x g for 2 minutes. Collect the flow-through that contains the sample. The recommended sample dilutions are as follows:
    - *Cell culture supernatant: 120 µl neat supernatant*
    - *Serum/Plasma: 2 µl serum/plasma in 100 µl Labeling Buffer*
    - *Cell/tissue lysate: 20 µg lysate in 100 µl Labeling Buffer*

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

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

B. Biotin-Labeling the Sample

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

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.
6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.
    a. For labeling cell culture supernatants: Add 8 µl of Labeling Reagent into the sample tube (for 120 µl supernatant).
    b. For labeling serum or plasma: Add 8 µl of Labeling Reagent into the sample tube (for 2 µl serum/plasma *in 100 µl labeling buffer*).
    c. For labeling cell or tissue lysates: Add 4 µl of 1X Labeling Reagent into the sample tube (for 20 µg lysate *in 100 µl labeling buffer*).
    d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

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

example in Step 6, adjust this volume proportionally.

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

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

C. Drying the Glass Slide

8. Remove the package containing the Assembled Glass Slide (Item E) from the freezer. Place unopened package on the bench top for ~15 minutes, and allow the Assembled Glass Slide to equilibrate to RT.
9. Open package, and take the Assembled Glass Slide out of the sleeve. Do <u>not</u> disassemble the Glass Slide from the chamber assembly. Place glass slide assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

   Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubations

Note: Glass slide should be <u>completely</u> dry before adding Blocking Buffer to wells.

10. Block sub-arrays by adding 400 µl of Blocking Buffer (Item F) into each well of Assembled Glass Slide and incubating at RT for 30 minutes. Ensure there are no bubbles on the array surfaces.
11. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 10-fold for cell culture supernatants, 20-fold for serum/plasma and 100-fold for cell/tissue lysate. *Dilution for other body fluid needs to be determined by the end user. Generally, most samples can be 10-20x dilution, while tears and saliva samples may need 100x dilution.*

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

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

    Note: Avoid the flow of sample into neighboring wells.

13. Based on number of samples and remaining protocol, calculate the amount of 1X Wash Buffer I and 1X Wash Buffer II needed to complete the experiment. Separately dilute the required amounts of 20X Wash Buffer I Concentrate (Item G) 20-fold and 20X Wash Buffer II Concentrate (Item H) with ddH_2O
14. Decant the samples from each well and wash 3 times with 800 µl of 1X Wash Buffer I at RT with gentle rocking or shaking for 5 minutes per wash.
15. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer I to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 10 minutes per wash.
16. Decant the Wash Buffer I from each well, place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer II to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 5 minutes per wash.
17. Prepare 1X Cy3-Conjugated Streptavidin:
    a. Briefly spin down tube containing the Cy3-Conjugated Streptavidin (Item I) immediately before use.
    b. Add 1000 µl of Blocking Buffer into the Cy3-Conjugated Streptavidin tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).
    c. To prepare 1X Cy3-Conjugated Streptavidin, add 200 µl of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800 µl of Blocking Buffer. Mix gently.
18. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400 µl of 1X Cy3-Conjugated Streptavidin to each

sub-array. Cover the incubation chamber with the plastic adhesive strips.

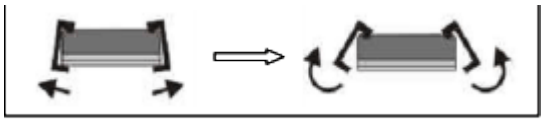
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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

    Note: Incubation may be done overnight at 4°C

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

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



21. Gently place the glass slide into 30 ml Centrifuge Tube (Item M). Add enough 1X Wash Buffer I to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 10 min. Remove the wash buffer. Repeat 2 times for a total of 3 washes.
22. Add enough 1X Wash Buffer II to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 5 minutes. Remove the wash buffer. Repeat one time for a total of two washes for 5 minutes per wash.
23. Finally, wash the glass slide with 30 ml of ddH_2O for 5 minutes. Remove glass slide and decant water from Centrifuge Tube.
24. Remove buffer droplets from the slide completely by one of the following ways:
    - Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
    - Or dry the glass slide by a compressed N2 stream.
    - Or gently apply suction with a pipette to remove buffer droplets. Do not touch the array surface, only the sides.

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

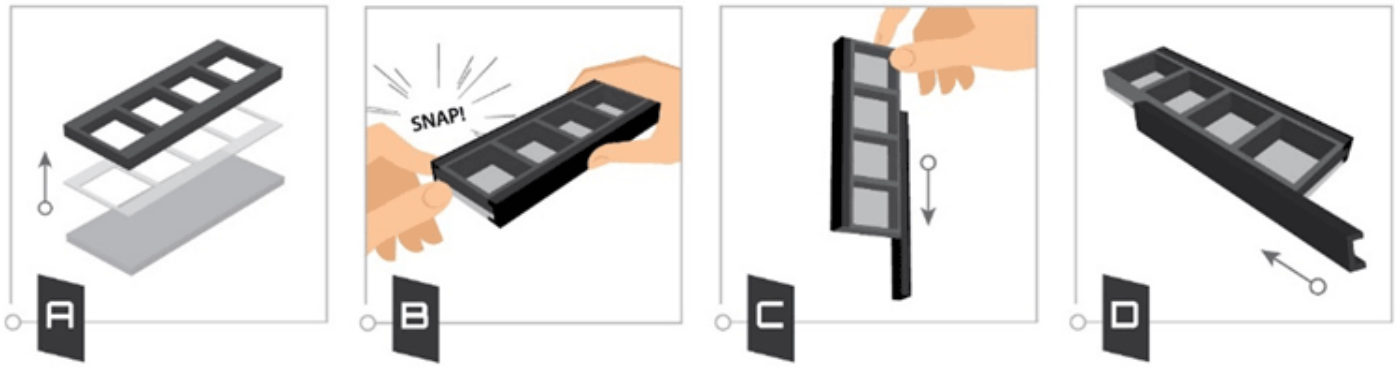
E. Fluorescence Detection

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

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

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

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



V. Antibody Array Map

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VI. Antibody Array Target List

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

VII. Interpretation of Results:

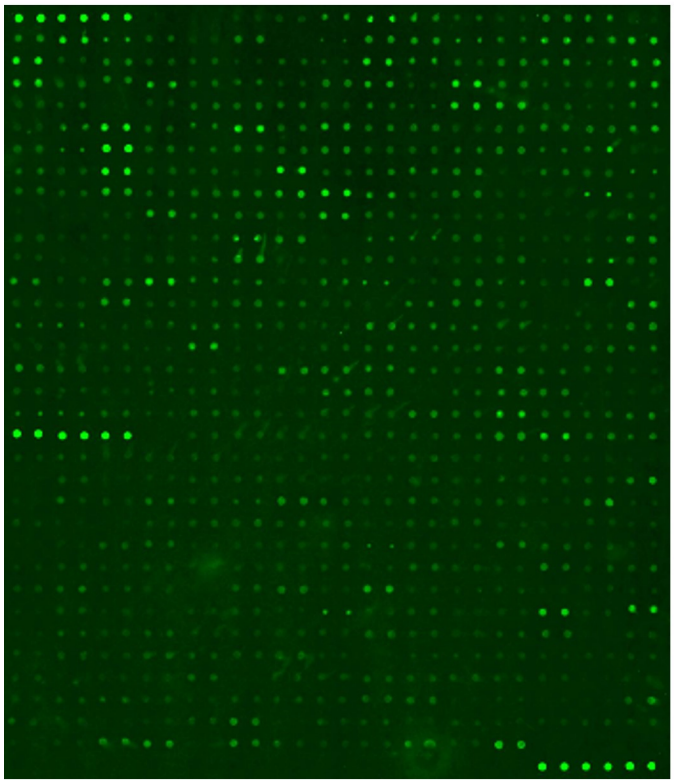
A. Explanation of Controls Spots

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

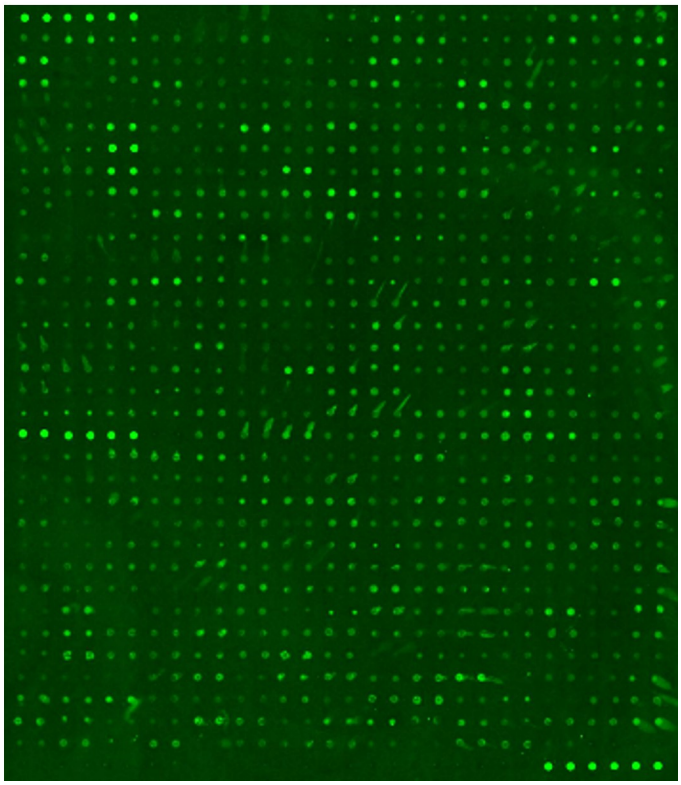
B. Typical Results

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

Serum



Plasma



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

C. Background Subtraction

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

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

D. Normalization of Array Data

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

You can calculate the normalized values as follows:

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

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

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

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

E. Threshold of Significant Difference

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

Any greater than or equal to 1.5-fold increase or less than or equal to 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 is around 95%).

VIII. Troubleshooting Guide

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

IX. Selected References

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