RayBio® Label-Based (L-Series) Human L9 Array, Glass Slide

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAH-BLG-9-4 (4 Sample Kit)
AAH-BLG-9-8 (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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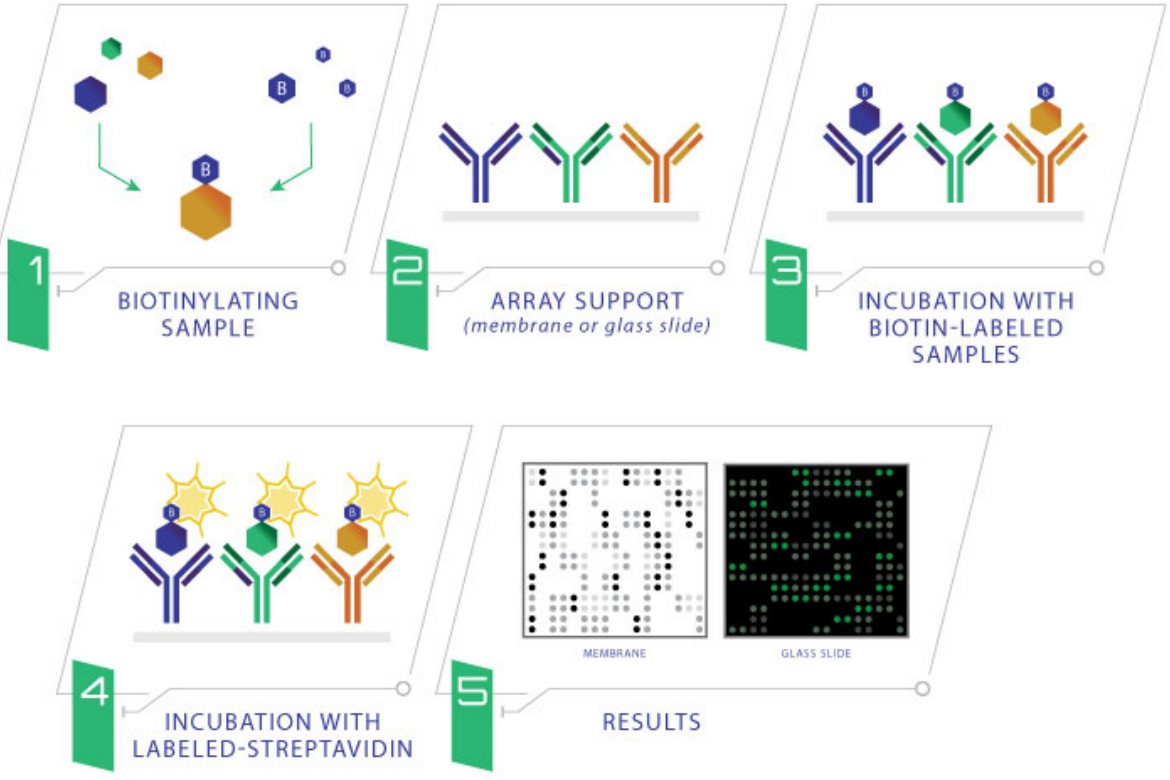
TABLE OF CONTENTS

I. Introduction

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

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



II. Materials Provided

A. Storage Recommendations

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

ITEM	DESCRIPTION	4 SAMPLE KIT	8 SAMPLE KIT
A	Spin Columns (0.5ml)	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.

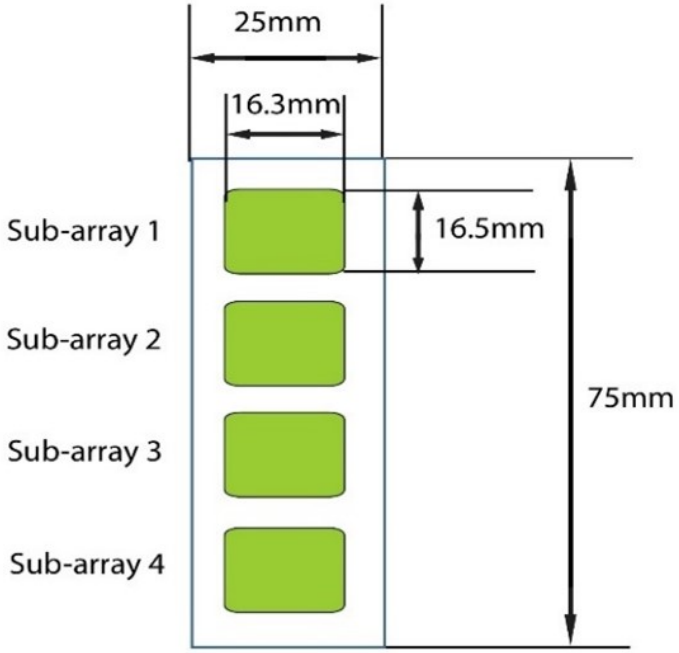
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

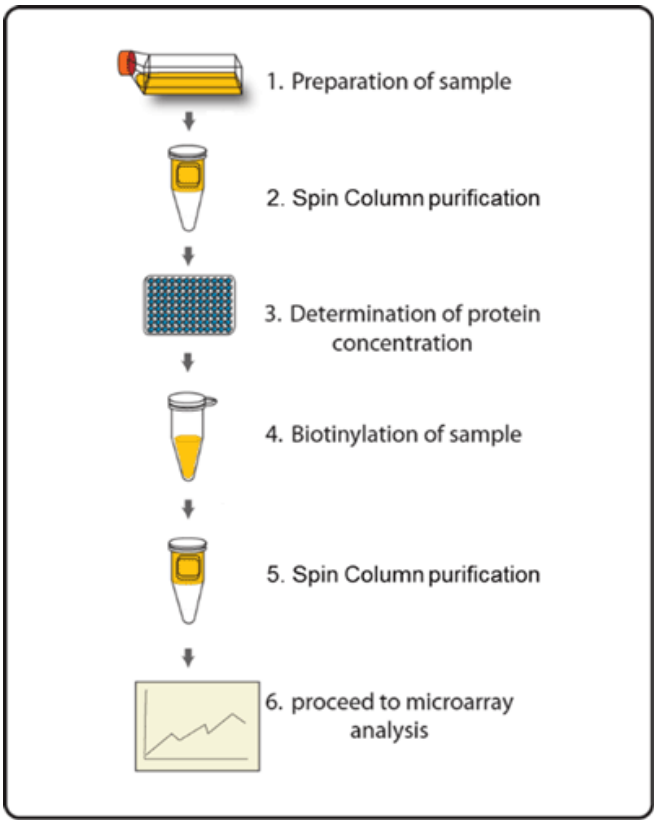
D. Incubations and Washes

- 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.

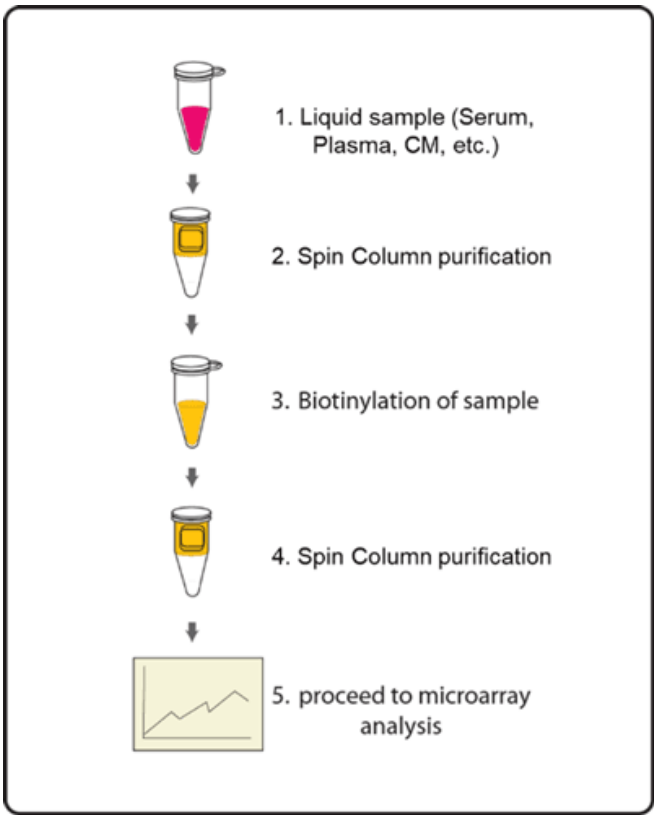
IV. Protocol

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.

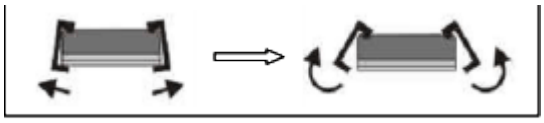
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

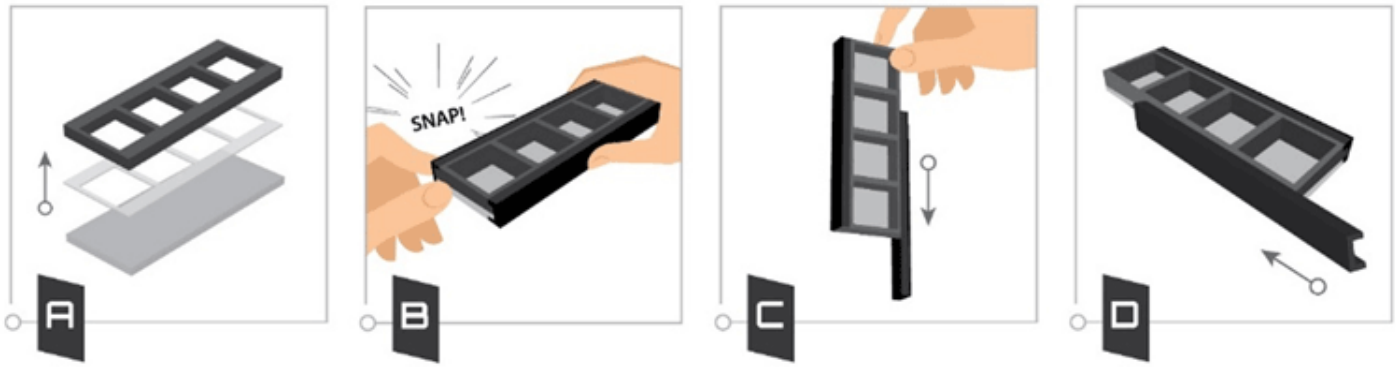
E. Fluorescence Detection

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	AARS2	73	CDK5RAP2	145	ELOVL5	217	HSD17B6	289	NSDHL	361	SH3GLB2	433	XYLB
2	ABCA10	74	CDK7	146	EMC2	218	HSPA1L	290	NT5C1A	362	SHISA5	434	YARS
3	ABCC11	75	CDKN1C	147	EMID1	219	HYI	291	NT5C3L	363	SIPA1L1	435	YARS2
4	ABCG5	76	CDYL2	148	ENOPH1	220	ICMT	292	NUDT12	364	SKIL	436	ZBTB11
5	ACOX3	77	CELA1	149	ENOSF1	221	IDH2	293	NUDT9	365	SLC1A2	437	ZFP14
6	ACTN3	78	CHAF1B	150	EPX	222	IFI27L2	294	NUP188	366	SLC25A16	438	ZFP57
7	ACTR3C	79	CHDH	151	ERCC8	223	IFNA7	295	NXF5	367	SLC27A6	439	ZFP92
8	ADAM30	80	CHERP	152	ERF	224	IGSF5	296	OLAH	368	SLC36A2	440	ZIK1
9	ADAR	81	CHMP4C	153	EVX1	225	IPKB	297	OR10C1	369	SLC36A3	441	ZNF154
10	ADARB2	82	CHRD	154	EVX2	226	IRG1	298	OR2AK2	370	SLC38A1	442	ZNF200
11	ADCY2	83	CHRNA4	155	EXOSC2	227	IRX1	299	OR2C3	371	SLC38A3	443	ZNF226
12	ADCY4	84	CHRNB2	156	EXOSC4	228	IRX2	300	OR4C15	372	SLC4A4	444	ZNF251
13	AER61	85	CHST10	157	FAM213	229	IRX3	301	OR4K1	373	SLC52A3	445	ZNF286A
14	AF10	86	CHST11	158	FANCC	230	JAZF1	302	OR52I2	374	SLC5A1	446	ZNF30
15	AGAP1	87	CHST12	159	FANCG	231	JPH1	303	OR5D13	375	SLC7A9	447	ZNF317
16	AHCYL1	88	CIB3	160	FANCL	232	KCNA1	304	OR5V1	376	SLC8A3	448	ZNF334
17	AHCYL2	89	CKMT2	161	FIS1	233	KCNC1	305	OR6K2	377	SLC9A3	449	ZNF404
18	AIG1	90	CLCN2	162	FKBP11	234	KCNG4	306	OR6T1	378	SLC9A7	450	ZNF41
19	AKAP11	91	CLDN23	163	FOXB1	235	KIAA1024	307	ORC3L	379	SLU7	451	ZNF417
20	AL2S7	92	CLK1	164	FOXI1	236	KIFC2	308	OXA1L	380	SLX1A	452	ZNF418
21	ALDH1A2	93	CLK4	165	FOXL1	237	KLF1	309	OXSM	381	SMAD6	453	ZNF419
22	ALDH1L2	94	CLNS1A	166	FPGS	238	LARS2	310	P4HA2	382	SMYD1	454	ZNF420
23	ALDH6A1	95	CMAS	167	FSCN2	239	LASS6	311	PAPSS1	383	SNRPB	455	ZNF431
24	ALX3	96	CNKSR2	168	FTCD	240	LIAS	312	PARD6A	384	SNX3	456	ZNF433
25	AMCASE	97	COL11A2	169	FXR1	241	LIG3	313	PARP4	385	SOAT2	457	ZNF440
26	AMD1	98	COL12A1	170	GABRA5	242	LIMK1	314	PC	386	SQLE	458	ZNF454
27	AMT	99	COL14A1	171	GABRB2	243	LPCAT2	315	PCBD2	387	SRR	459	ZNF486
28	ANGEL1	100	COL4A4	172	GABRE	244	LRRC37A3	316	PDE1A	388	SRRM1	460	ZNF517
29	ANKRA2	101	COL5A2	173	GABRQ	245	MAD4	317	PDHX	389	SSR4	461	ZNF527
30	AP1G2	102	COL7A1	174	GABRR2	246	MAGEL2	318	PDXK	390	ST3GAL4	462	ZNF529
31	APOL4	103	COQ2	175	GAL3ST3	247	MAN2A2	319	PECR	391	STX17	463	ZNF534
32	APOL5	104	COX10	176	GAL3ST4	248	MAN2B2	320	PELO	392	STX18	464	ZNF546
33	APOL6	105	COX11	177	GALM	249	MANBA	321	PGM2	393	SUCLA2	465	ZNF548
34	APRT	106	COX17	178	GALNT12	250	MAP3K1	322	PGM2L1	394	SUCLG2	466	ZNF554
35	ARHGAP17	107	CPSF2	179	GALNTL4	251	MAP3K9	323	PI4K2B	395	SUV39H1	467	ZNF560
36	ART1	108	CRYL1	180	GCAT	252	MARK4	324	PIGG	396	SUV39H2	468	ZNF561
37	ATP12A	109	CSAD	181	GCLM	253	MAST2	325	PIGP	397	SUV420H1	469	ZNF562
38	ATP6V0A1	110	CSGALNACT2	182	GDPD3	254	MAT1A	326	PINX1	398	SV2B	470	ZNF564
39	ATP6V1G2	111	CSTF3	183	GGCX	255	MCCC2	327	PKIA	399	SV2C	471	ZNF565
40	ATP7A	112	CTRB1	184	GGT2	256	ME2	328	PKN3	400	TACR3	472	ZNF566
41	ATP8B2	113	CTRL	185	GGT7	257	MEF2D	329	PKP2	401	TAPBP	473	ZNF567
42	ATXN7	114	CUEDC1	186	GLRB	258	MGAT4A	330	PNPO	402	TBL3	474	ZNF570
43	AUH	115	CUL4B	187	GLYAT	259	MIOX	331	POFUT1	403	TFDP2	475	ZNF582
44	AVEN	116	CYB5R1	188	GMPPA	260	MRI1	332	POLR3E	404	THOC1	476	ZNF583
45	B4GALNT1	117	CYB5R2	189	GMPR	261	MRPL1	333	POLR3H	405	THOC3	477	ZNF584
46	B4GALNT3	118	CYB5R3	190	GMPR2	262	MRPL20	334	POU3F1	406	THOC7	478	ZNF587
47	BBS1	119	CYBA	191	GNA14	263	MRPL23	335	PPAT	407	THTPA	479	ZNF607
48	BCKDHA	120	CYP21A2	192	GNPDA1	264	MRPL24	336	PPP1R3A	408	TKTL1	480	ZNF610
49	BNIP1	121	CYP39A1	193	GNPDA2	265	MRPL34	337	PRDM12	409	TMLHE	481	ZNF624
50	BTG2	122	CYTH4	194	GNPNAT1	266	MRPL9	338	PRDM9	410	TRIM37	482	ZNF643
51	BTG4	123	DCPS	195	GORAB	267	MRPS10	339	PTAFR	411	TRPC5	483	ZNF674
52	BTNL9	124	DCTD	196	GPRC6A	268	MRPS21	340	PTTG2	412	TUBB6	484	ZNF675
53	BUD31	125	DDX46	197	GPT2	269	MTHFD1	341	PWP2	413	UAP1L1	485	ZNF676
54	C1QTNF2	126	DERL2	198	GRIA1	270	MTHFD1L	342	QARS	414	UBA6	486	ZNF680
55	C1QTNF7	127	DHCR24	199	GRIN2D	271	MTHFD2	343	RAB39B	415	UBE2J2	487	ZNF682
56	C21orf58	128	DHDH	200	GTF2F2	272	MTHFS	344	RAD1	416	UBE4A	488	ZNF689
57	C3HC4	129	DHRS3	201	GUK1	273	MTMR2	345	RAD9A	417	UBE4B	489	ZNF709
58	CABLES2	130	DHTKD1	202	HADH	274	MVD	346	RAG1	418	UCK1	490	ZNF714
59	CACNA1G	131	DLAT	203	HADHB	275	MYBL2	347	RASGRF1	419	UCKL1	491	ZNF764
60	CACNA2D4	132	DNAJB12	204	HERC5	276	MYH4	348	RBP2	420	UCN2	492	ZNF765
61	CACNB2	133	DPF1	205	HIBCH	277	MYL6B	349	RCAN1	421	UCRC	493	ZNF778
62	CACNG6	134	DPM1	206	HIST2H2AA3	278	MYLK4	350	RFC3	422	UFD1L	494	ZNF780B
63	CACNG8	135	DPM2	207	HIST2H2AC	279	NAGPA	351	RFC4	423	UMPS	495	ZNF785
64	CACYBP	136	DTYMK	208	HLA-DPA1	280	NANS	352	RNASEL	424	VAMP4	496	ZNF91
65	CaMK1b	137	DUSP8	209	HLA-DQA2	281	NARS2	353	RNGTT	425	VASP	497	ZNF98
66	CAMKK2	138	DUT	210	HLCS	282	ND5	354	RPA3	426	VAV3	498	ZP1
67	CARD18	139	DYNC1I1	211	HMBS	283	ND6	355	RPL18A	427	VMP1	499	ZSCAN1
68	CBX6	140	DYRK1B	212	HMGCL	284	NFATC4	356	RPL23A	428	VPS37B	500	ZXDA
69	CDC16	141	EBP	213	HMGCS1	285	NMNAT3	357	SEMA3D	429	WDR33		
70	CDC26	142	EIF2B3	214	HOGA1	286	NNT	358	SEPHS2	430	WIBG		
71	CDC34	143	ELMO3	215	HS3ST3A1	287	NPAS2	359	SETD1B	431	WIPI1		
72	CDCA4	144	ELOA3	216	HSD17B4	288	NRBF2	360	SETD2	432	XRCC3		

VII. Interpretation of Results:

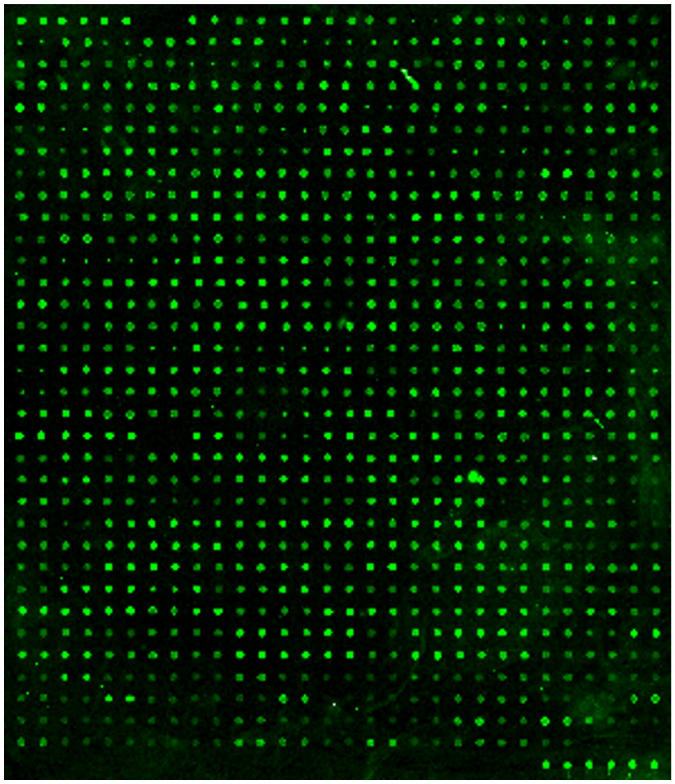
A. Explanation of Controls Spots

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.

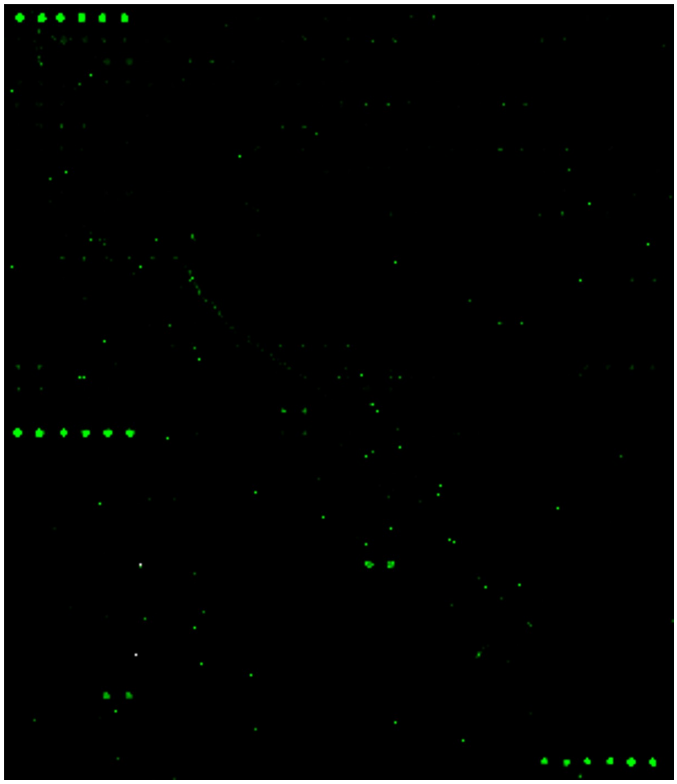
B. Typical Results

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.

Human Serum



Buffer Control



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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