RayBio® Label-Based (L-Series) Human L11 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-11-4 (4 Sample Kit)
AAH-BLG-11-8 (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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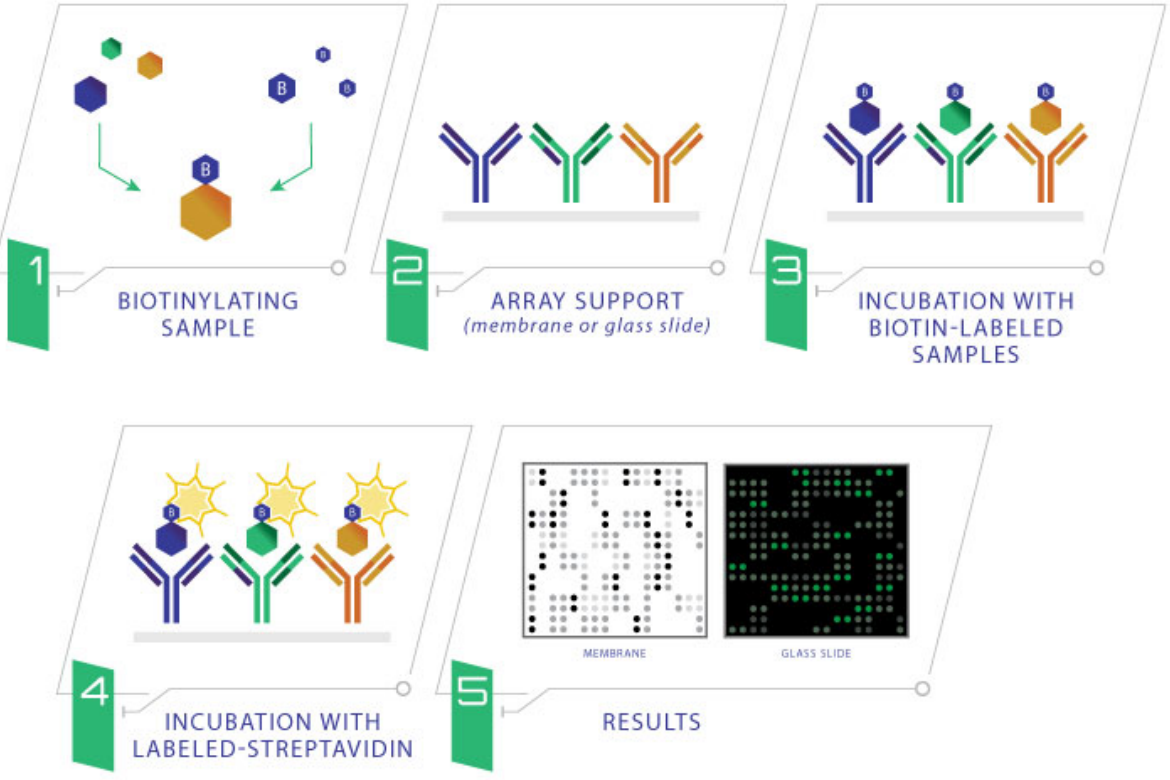
I. Introduction

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.

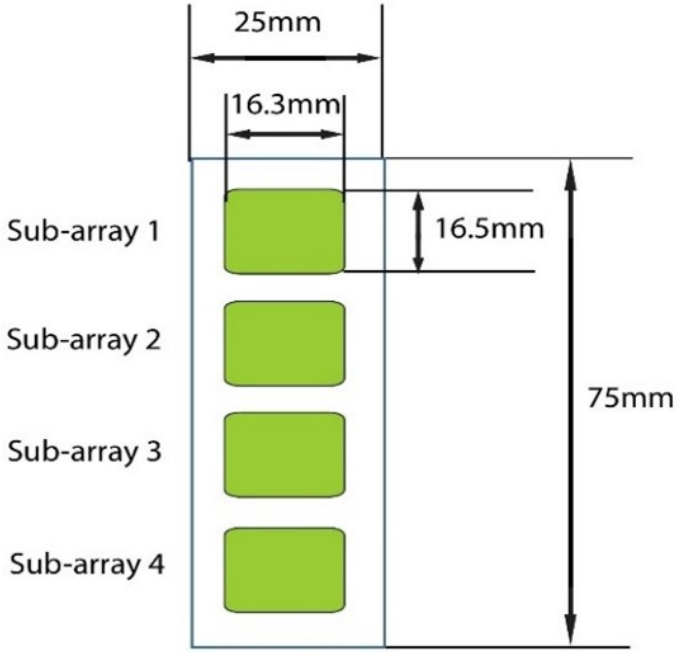
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

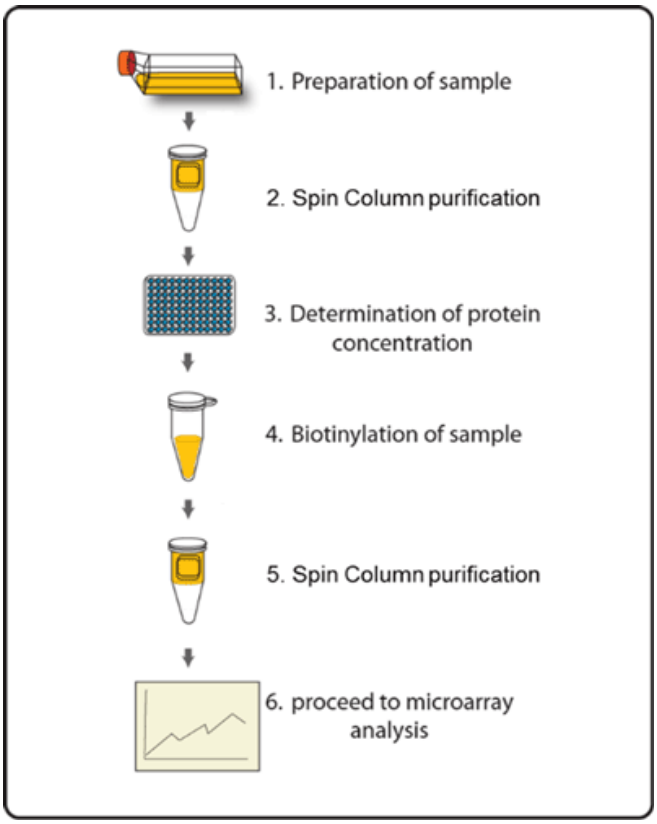
D. Incubations and Washes

- 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.

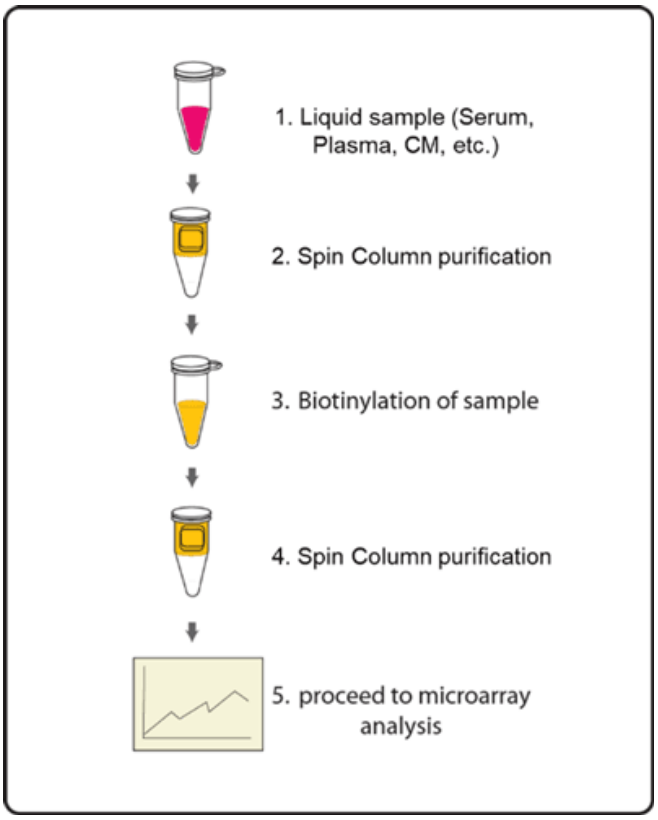
IV. Protocol

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.

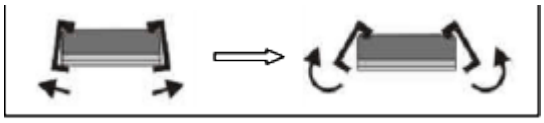
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

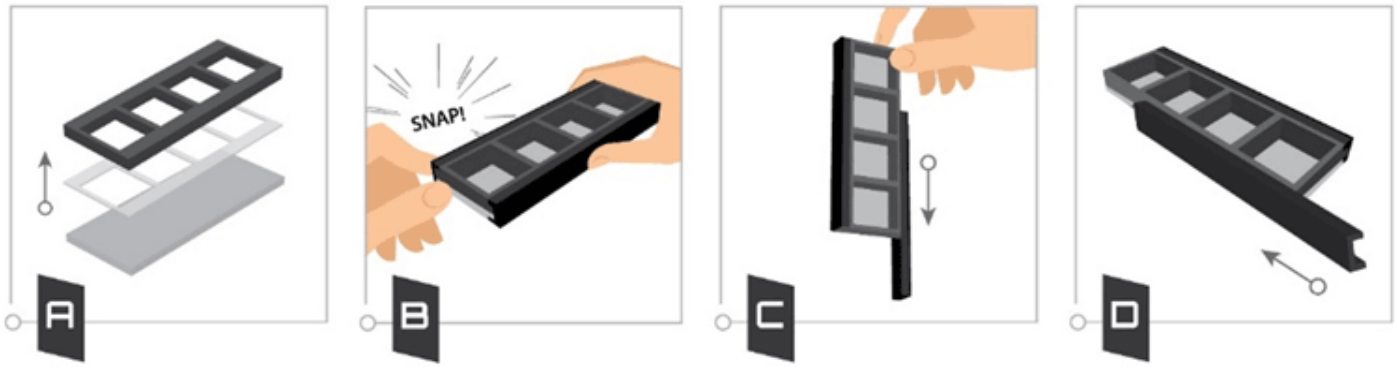
E. Fluorescence Detection

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	ABCC13	73	ATP6AP1	145	COL11A1	217	GEM	289	KLC1	361	POLR2E	433	STON1
2	ABHD2	74	ATP6V1A	146	COL1A2	218	GJA4	290	KLHL1	362	POLR2G	434	SULT1C4
3	ACR	75	ATP6V1B1	147	COL26A1	219	GJC1	291	KLHL2	363	POLR2J	435	SURF6
4	ACTL7A	76	ATP6V1E2	148	COL4A5	220	GJC3	292	KPNA3	364	POLR2J2	436	SYT2
5	ACTL7B	77	ATP6V1G1	149	COL8A2	221	GNAI2	293	KPNA5	365	POLR2K	437	SYTL1
6	ACTR10	78	ATP6V1G3	150	CORO1A	222	GNAL	294	KRT25	366	POU4F3	438	TASP1
7	ACTR1A	79	ATP6V1H	151	CORO2B	223	GNG12	295	KRT27	367	PPEF1	439	TBC1D10A
8	ACTR1B	80	ATP8	152	COX18	224	GNG5	296	KRT32	368	PPFIBP2	440	TBC1D21
9	ACTRT2	81	ATP8A1	153	COX6A1	225	GNG8	297	KRT37	369	PPP1R16A	441	TCF19
10	ADAMTS2	82	ATX3	154	COX6A2	226	GPR103	298	L3MBTL1	370	PPP1R3B	442	TCFL5
11	ADAP2	83	AURKC	155	COX7A1	227	GPR114	299	LCA5L	371	PPP4R1L	443	TEF
12	ADAR2	84	AXUD1	156	COX7A2L	228	GPR123	300	LGALS13	372	PRDM15	444	TESK1
13	ADCY1	85	BAIAP2	157	COX7B	229	GPR143	301	LRP3	373	PRKY	445	TFAP2A
14	ADCY3	86	BARHL2	158	COX7B2	230	GPR144	302	MAP126	374	PRRX2	446	TLE4
15	ADCY6	87	BAZ2A	159	COX7C	231	GPR151	303	MAZ	375	PSKH1	447	TOMM40
16	ADCY8	88	BBS7	160	COX8A	232	GPR152	304	MEF2A	376	PSMD11	448	TRIM2
17	ADCY9	89	BCO2	161	CPA3	233	GPR160	305	MKRN1	377	PSMD3	449	TRIM24
18	ADGRB2	90	BEST3	162	CPA5	234	GPR174	306	MNT	378	PSMD8	450	TRIM45
19	ADGRG4	91	BIN3	163	CPA6	235	GPR18	307	MPHOSPH9	379	PTPRN2	451	TSKS
20	ADIPOR2	92	BLOC1S5	164	CPNE4	236	GPR20	308	MPRG	380	RAB29	452	TSPYL1
21	AGFG2	93	BMP13	165	CPSF4	237	GPR25	309	MRGPRX1	381	RAB30	453	TSSK2
22	AKAP17A	94	BNC1	166	CPT1A	238	GPR27	310	MRPS30	382	RAB36	454	TUBA3C
23	AKAP5	95	BRP44L	167	CPT1B	239	GPR4	311	MTFP1	383	RAG2	455	TUBAL3
24	ALOXE3	96	BTBD6	168	CRCP	240	GPR62	312	MTND3	384	RASD2	456	TUBGCP5
25	ALY	97	C4A	169	CRSP7	241	GPR83	313	MYBL1	385	REST	457	TUBGCP6
26	AMBN	98	CABLES1	170	CRYBA1	242	GPR85	314	MYH15	386	RFTN1	458	U2AF1
27	AMBRA1	99	CABP4	171	CRYBB3	243	GPR87	315	MYH8	387	RGS16	459	UBE2D2
28	ANAPC1	100	CACNA1C	172	CSH2	244	GPRIN2	316	MYL10	388	RGS4	460	UBE2G2
29	ANAPC2	101	CACNA1E	173	CSN3	245	GPX7	317	MYL6	389	RHOH	461	ULK2
30	ANAPC4	102	CACNA1S	174	CTDSP1	246	GRK4	318	MYO1C	390	RLBP1	462	UQCRFS1
31	ANKRD2	103	CACNG5	175	CTNNAL1	247	GSG2	319	MYO3B	391	RNASE3	463	UQCRQ
32	ANO3	104	CAPN11	176	CX32	248	GUCY1B2	320	MYO6	392	RNF113B	464	URI1
33	ANP32D	105	CAPN12	177	CYC1	249	GYS1	321	MYOM2	393	RNF130	465	USP35
34	AP2A1	106	CBLN3	178	DAB1	250	HDC	322	MZF1	394	RNF144A	466	WDR21A
35	AP2M1	107	CCNI	179	DCLRE1C	251	HES2	323	N4BP1	395	RNF19A	467	YAF2
36	AP2S1	108	CCT6B	180	DHRS7	252	HIRA	324	NDUFA1	396	RPH3A	468	YBX2
37	APBA1	109	CD180	181	DIAPH1	253	HIST1H2AH	325	NDUFA12	397	RRAD	469	ZBED1
38	APBB2	110	CDC2L6	182	DIRAS1	254	HKR1	326	NDUFA4	398	RRH	470	ZC3H7B
39	APBB3	111	CDH18	183	DMAP1	255	HLA-DMA	327	NDUFA7	399	RRP1B	471	ZDHHC9
40	ARHGAP10	112	CDH7	184	DNAI2	256	HLA-DMB	328	NDUFAB1	400	SAR1B	472	ZFP36
41	ARHGAP26	113	CDK10	185	DNTTIP1	257	HLA-DOA	329	NDUFAF4	401	SCAND1	473	ZHX2
42	ARHGAP9	114	CDK5RAP1	186	DONSON	258	HLA-DOB	330	NDUFS1	402	SCN8A	474	ZKSCAN3
43	ARHGEF2	115	CDKL5	187	DTNA	259	HLA-DQB2	331	NDUFS4	403	SCNN1A	475	ZMIZ2
44	ARL4C	116	CDKN3	188	DTNB	260	HLA-DRB3	332	NDUFS8	404	SCNN1D	476	ZMYND11
45	ARL5B	117	CDR2	189	DYNLRB2	261	HOXA3	333	NDUFV1	405	SENP6	477	ZNF133
46	ARPC4	118	CEBPD	190	EIF2B5	262	HOXA4	334	NEK4	406	SEPTIN7	478	ZNF148
47	ARPP21	119	CENPM	191	EIF4G2	263	HOXA7	335	NFAT5	407	SERPINB13	479	ZNF169
48	ARR3	120	CEP110	192	ELMO1	264	HOXB2	336	NFYB	408	SFRS9	480	ZNF225
49	ARRB2	121	CEP135	193	EML1	265	HOXB4	337	NGB	409	SGCB	481	ZNF227
50	ARSD	122	CEP152	194	EMX1	266	HOXC11	338	NKX2-4	410	SGLT2	482	ZNF25
51	ART5	123	CEP63	195	EPM2AIP1	267	HOXC12	339	NOX1	411	SHOX	483	ZNF337
52	ARVCF	124	CEP72	196	EXOC7	268	HOXC13	340	NR2F1	412	SIM1	484	ZNF397
53	AS160	125	CEP76	197	FAM120C	269	HOXC6	341	NUDT6	413	SIX5	485	ZNF398
54	ASB4	126	CHD2	198	FAM13A	270	HPS6	342	NULP1	414	SLC1A1	486	ZNF436
55	ASB8	127	CHRM1	199	FASTK	271	INCENP	343	OAS1	415	SLC22A17	487	ZNF442
56	ATE1	128	CHRNA6	200	FBG3	272	INSIG1	344	OPN3	416	SLC25A21	488	ZNF519
57	ATF3	129	CIITA	201	FBG4	273	IPO13	345	OPN4	417	SLC25A31	489	ZNF540
58	ATF7	130	CKLF1	202	FBXL20	274	IQCB1	346	OPRM1	418	SLC25A5	490	ZNF592
59	ATP11B	131	CKLF2	203	FBXO24	275	ITGA7	347	OR4A4P	419	SLC28A1	491	ZNF596
60	ATP13A1	132	CKLF4	204	FCGR2C	276	KATNB1	348	OSBPL11	420	SLC28A2	492	ZNF7
61	ATP1A1	133	CLCN1	205	FKLF	277	KCNA3	349	OSBPL6	421	SLC4A9	493	ZNF707
62	ATP1A2	134	CLCN4	206	FKSG2	278	KCNAB1	350	OSGIN2	422	SLC6A14	494	ZNF76
63	ATP2A3	135	CLCN6	207	FOXD4	279	KCNAB3	351	PAX9	423	SLC6A15	495	ZNF763
64	ATP2C1	136	CLCNKA	208	FOXD4L1	280	KCNB1	352	PDE4C	424	SLC6A17	496	ZNF786
65	ATP5C1	137	CLDN14	209	FOXN1	281	KCNJ3	353	PDP2	425	SLC6A8	497	ZNF792
66	ATP5F1	138	CLDN2	210	FSBP	282	KCNJ5	354	PHACTR3	426	SLC9A3R2	498	ZNF83
67	ATP5G1	139	CLDN9	211	FTSJ1	283	KCNJ9	355	PHEX	427	SLC9A9	499	ZP4
68	ATP5G2	140	CLN3	212	FUS2	284	KCNK15	356	PIK3R6	428	SNTA1	500	ZRANB1
69	ATP5G3	141	CLTB	213	GABARAPL1	285	KCNN4	357	PKCB1	429	SOX13		
70	ATP5J	142	CMTM3	214	GABPB1	286	KDELR2	358	PLCB2	430	SP2		
71	ATP5S	143	CNTN6	215	GAS2L1	287	KIAA0494	359	PLCZ1	431	SPG7		
72	ATP6	144	COG8	216	GCKR	288	KIR2DL2	360	PLEKHA3	432	SREBF1		

VII. Interpretation of Results:

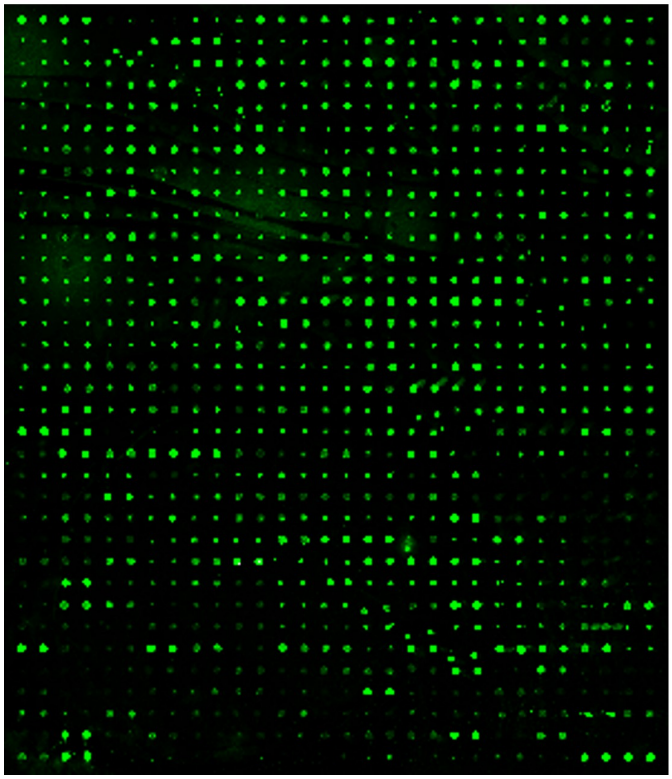
A. Explanation of Controls Spots

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.

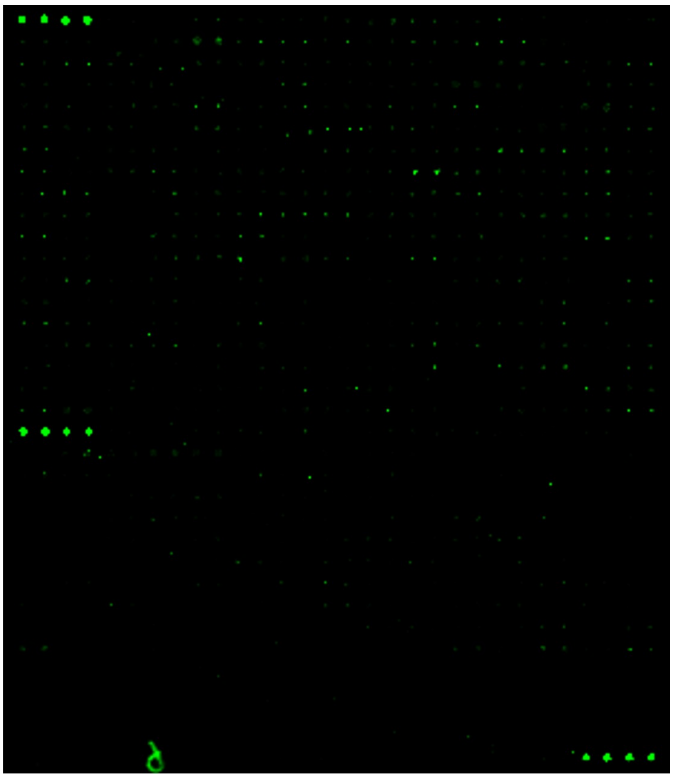
B. Typical Results

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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