RayBio® Label-Based (L-Series) Human L6 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-6-4 (4 Sample Kit)
AAH-BLG-6-8 (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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Tel: +1-770-729-2992 or 1-888-494-8555 (Toll Free); Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

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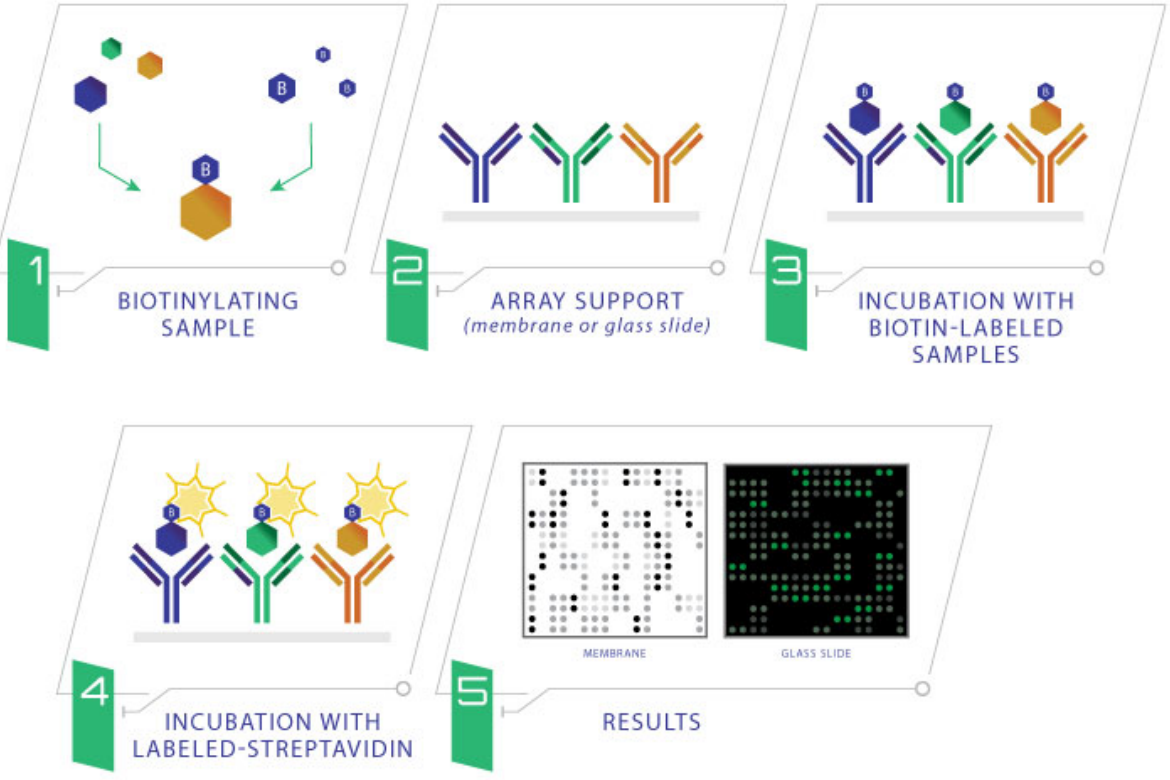
I. Introduction

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.

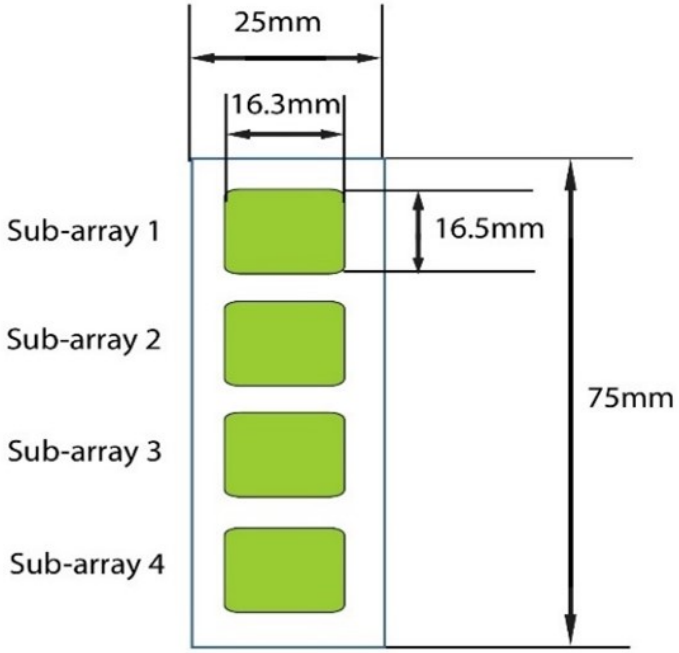
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

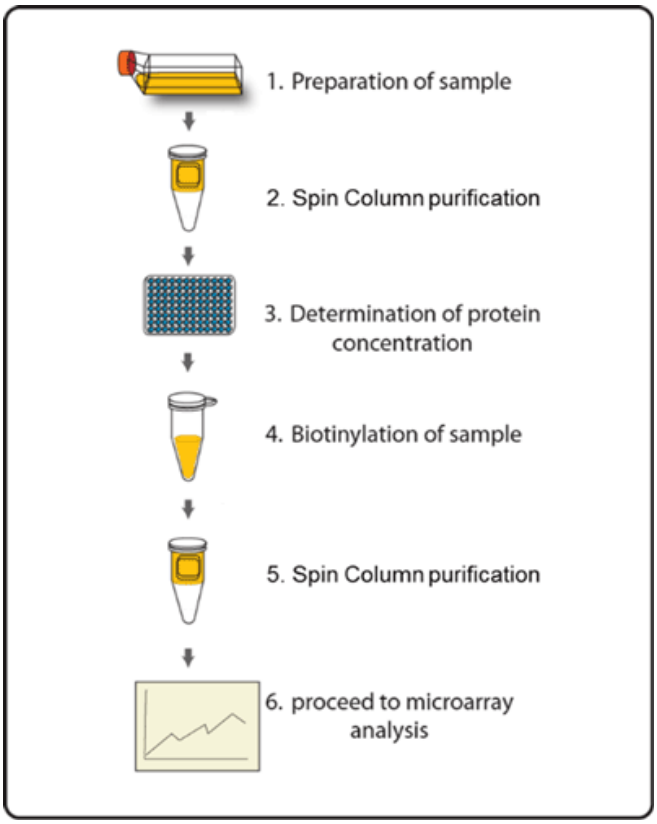
D. Incubations and Washes

- 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.

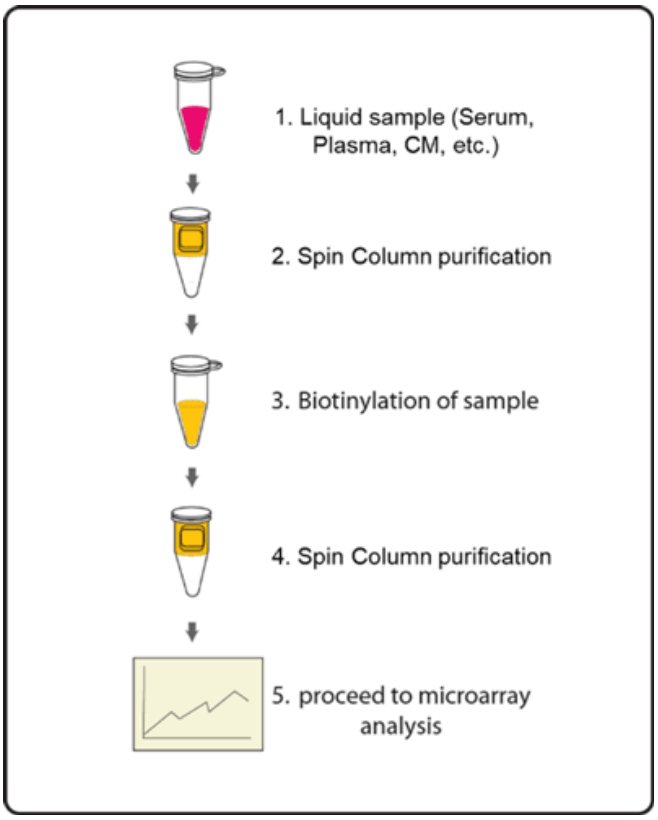
IV. Protocol

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.

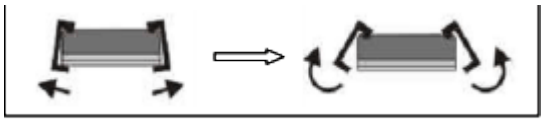
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

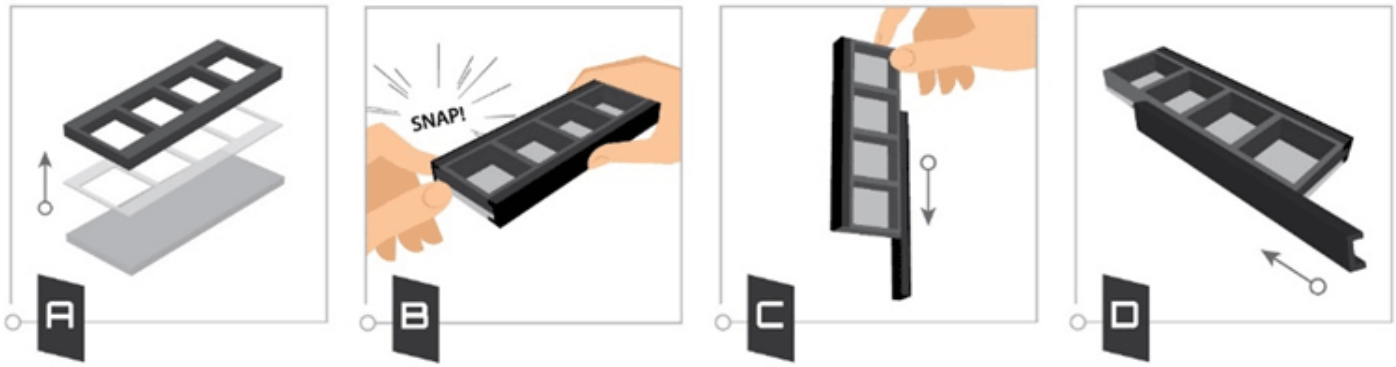
E. Fluorescence Detection

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	AADAC	73	CA10	145	DEFB118	217	IFT81	289	NUDT2	361	RASA1	433	SYNGR3
2	AARS1	74	CABP5	146	DEPTOR	218	IGHMBP2	290	OFD1	362	RBPMS	434	SYS1
3	AARSD1	75	CACNB3	147	DFFB	219	IGLL1	291	OPHN1	363	RFXANK	435	SYT11
4	ABCB5	76	CACNG1	148	DHRSX	220	ING5	292	OPTN	364	RGL4	436	TAOK3
5	ABI3	77	CACNG4	149	DIAPH2	221	IRAK4	293	OSBPL3	365	RGS5	437	TARBP2
6	ACHE	78	CALCR	150	DLGAP2	222	IRX4	294	OTOA	366	RGS6	438	TBC1D14
7	ADAM20	79	CALCRL	151	DLX6	223	IRX6	295	OTUB2	367	RHCE	439	TCF4
8	ADGRF3	80	CAMLG	152	DMBT1	224	IVD	296	PAK4	368	RHOA	440	TESK2
9	ADRA1B	81	CAPN3	153	DNAJC12	225	KANK1	297	PAK6	369	RMND5B	441	TET3
10	ADRA2A	82	CARD11	154	DRP2	226	KARS1	298	PALM	370	RNF11	442	TEX13B
11	ADRA2B	83	CARM1	155	DTX1	227	KCNA6	299	PANX1	371	RNF112	443	TEX264
12	ADRB2	84	CASQ1	156	DZIP3	228	KCNC3	300	PAX8	372	RNF2	444	TGIF2LX
13	ADRB3	85	CASZ1	157	E2F4	229	KCNG1	301	PAXBP1	373	RNF217	445	THAP6
14	AFTPH	86	CCDC134	158	E2F6	230	KCNH7	302	PCDH17	374	RNF26	446	TIMM10B
15	AGGF1	87	CCDC8	159	EBAG9	231	KCNIP3	303	PCDH18	375	ROBO1	447	TIMM17A
16	AGO4	88	CCDC9	160	EDN3	232	KCNS3	304	PCDHB3	376	ROM1	448	TIMM17B
17	AGTR2	89	CCDC91	161	EEFSEC	233	KIAA0355	305	PCIF1	377	RPGR	449	TMC2
18	AHCY	90	CCNA1	162	EGR4	234	KIF25	306	PCMTD2	378	RRAS2	450	TMCC2
19	AIMP1	91	CCNO	163	EHMT1	235	KIFAP3	307	PCP4	379	RRM1	451	TMED10
20	AKAP10	92	CD19	164	EHMT2	236	KIFC1	308	PDCD5	380	RRM2B	452	TMEM199
21	AKAP7	93	CD200R1L	165	EIF4E	237	KIR2DL1	309	PDCD6	381	RTN1	453	TMEM25
22	ALDH3A1	94	CD37	166	EIF5	238	KLHL17	310	PDE2A	382	RXFP1	454	TNFAIP8
23	ALDH7A1	95	CD3D	167	ELL2	239	KLHL32	311	PFDN2	383	S100A3	455	TNIP1
24	ALKBH1	96	CD72	168	EMC8	240	KLK4	312	PFDN4	384	SAE1	456	TNM2
25	ALPI	97	CDH8	169	ENDOU	241	L3MBTL2	313	PHF2	385	SCML2	457	TNM4
26	ALS2CR12	98	CDK11B	170	EPDR1	242	LHX6	314	PHOSPHO1	386	SEC13	458	TNRC6A
27	AMACR	99	CDK13	171	ERBIN	243	LILRB3	315	PHYH	387	SEC14L1	459	TRAIP
28	AMH	100	CDK3	172	ERN2	244	LNX2	316	PIAS4	388	SECISBP2L	460	TRAK1
29	AMMECR1	101	CDKN2D	173	ETFDH	245	LRFN5	317	PIN1	389	SELPLG	461	TRIAP1
30	ANKH	102	CDON	174	ETHE1	246	LRRC29	318	PITPNA	390	SEMA3F	462	TRIM23
31	ANKMY1	103	CDX1	175	ETV3	247	LSM1	319	PIWIL2	391	SENP8	463	TRIM31
32	ANXA3	104	CDYL	176	EXOC6	248	LYPD6	320	PKN2	392	SERINC2	464	TRIM7
33	ANXA9	105	CEBPZ	177	FAM161B	249	MAP2K2	321	PLCB4	393	SERPINB9	465	TSC1
34	APBB1IP	106	CEND1	178	FAM43B	250	MAP2K5	322	PLEKHA2	394	SERPINI2	466	TSC22D1
35	APTX	107	CFAP410	179	FAM83C	251	MAPK14	323	PLSCR4	395	SETD7	467	TSEN34
36	ARIH2	108	CHEK1	180	FAM9B	252	MBD3L1	324	PMVK	396	SFXN2	468	TSPAN7
37	ARL4A	109	CHEK2	181	FANCA	253	MED28	325	PNLIP	397	SIAE	469	TTC14
38	AS3MT	110	CHRM2	182	FBXL7	254	MEOX2	326	POLG	398	SIAH2	470	TUB
39	ASS1	111	CHRNB3	183	FBXO11	255	MERIT40	327	POLG2	399	SIN3B	471	TUBA1A
40	ATF2	112	CIB2	184	FBXO27	256	MID1IP1	328	PPCDC	400	SIX6	472	UBE2D1
41	ATG4B	113	CKAP4	185	FBXO28	257	MKI67	329	PPIG	401	SLBP	473	UBE2D4
42	ATP5D	114	CLDN8	186	FBXO34	258	MKKS	330	PPIL1	402	SLC12A1	474	UBR5
43	ATP6V0A4	115	CLN6	187	FBXO7	259	MLPH	331	PPIL2	403	SLC13A1	475	UBXN2A
44	ATP6V0C	116	CLOCK	188	FBXW7	260	MMAA	332	PPM1B	404	SLC25A2	476	USH1C
45	ATP6V1B2	117	CLPX	189	FCER1G	261	MOAP1	333	PPM1G	405	SLC35A2	477	USP13
46	ATP6V1C1	118	CMPK1	190	FFAR2	262	MOB1B	334	PPM1L	406	SLC35A3	478	USP36
47	ATP6V1C2	119	CNKSR1	191	FKBP6	263	MPP2	335	PPP1R10	407	SLC6A18	479	USP38
48	ATP6V1D	120	CNR1	192	FKBP7	264	MRPL45	336	PPP1R8	408	SLIT2	480	VANGL2
49	ATP6V1E1	121	CNR2	193	GAS2	265	MRPS25	337	PPP1R9B	409	SMPDL3A	481	WASL
50	ATP8B4	122	COG3	194	GAS7	266	MSH5	338	PRDM5	410	SNAPC2	482	WNT2B
51	ATRIP	123	COL4A3BP	195	GCNT1	267	MSRB2	339	PRKAB1	411	SNIP1	483	WNT7A
52	ATXN7L1	124	COPZ2	196	GFPT1	268	MTFR1	340	PRKAR1B	412	SNPH	484	WNT9B
53	AUP1	125	COQ7	197	GGPS1	269	MTRR	341	PRKCB	413	SNX8	485	XK
54	BAG4	126	COQ8A	198	GPATCH2	270	MYBPC1	342	PRKCG	414	SPINK4	486	ZBTB20
55	BBOX1	127	CORO2A	199	GPR150	271	MYL2	343	PRKD3	415	SPPL2A	487	ZCCHC4
56	BBS2	128	COX5B	200	GPR19	272	MYO7A	344	PROKR2	416	SRCIN1	488	ZFHX2
57	BCAP29	129	CPLX2	201	GPR63	273	MYT1	345	PRTFDC1	417	SRFBP1	489	ZIM2
58	BCAP31	130	CPNE1	202	GRAP	274	MZB1	346	PSG1	418	SRI	490	ZNF219
59	BCKDK	131	CRHR1	203	GRB14	275	N4BP2	347	PSG6	419	SRPK1	491	ZNF232
60	BHLHE41	132	CRIP2	204	GRK7	276	NCF2	348	PSPH	420	ST3GAL2	492	ZNF280A
61	BLZF1	133	CRY1	205	GSTT2B	277	NCKIPSD	349	PTGFRN	421	STAC	493	ZNF280B
62	BOK	134	CSRP3	206	H2AFY2	278	NEMP1	350	PTP4A2	422	STK17B	494	ZNF282
63	BPI	135	CTHRC1	207	HAS1	279	NFIB	351	PTPN9	423	STK31	495	ZNF384
64	BRF1	136	CTNNBIP1	208	HDAC4	280	NLGN3	352	PTPRU	424	STX1B	496	ZNF410
65	BRIP1	137	CYB561	209	HEMK1	281	NLK	353	PTS	425	STX3	497	ZNF483
66	BSND	138	CYGB	210	HES6	282	NLRP5	354	RAB11A	426	STX5	498	ZNF512B
67	BST2	139	CYP7A1	211	HLA-DQA1	283	NMT2	355	RAB27A	427	STXBP1	499	ZP2
68	BTBD9	140	DACH1	212	HOXB9	284	NPHP1	356	RAB5IF	428	SUB1	500	ZWINT
69	BUB1	141	DARS1	213	HPCA	285	NRAS	357	RABGEF1	429	SULT1A1		
70	BUB3	142	DCDC2	214	HSF1	286	NRG4	358	RAD18	430	SUN1		
71	C1D	143	DCTPP1	215	IFNA4	287	NSL1	359	RAP1A	431	SUN2		
72	C20orf96	144	DDHD1	216	IFT122	288	NUBPL	360	RAP2A	432	SYN3		

VII. Interpretation of Results:

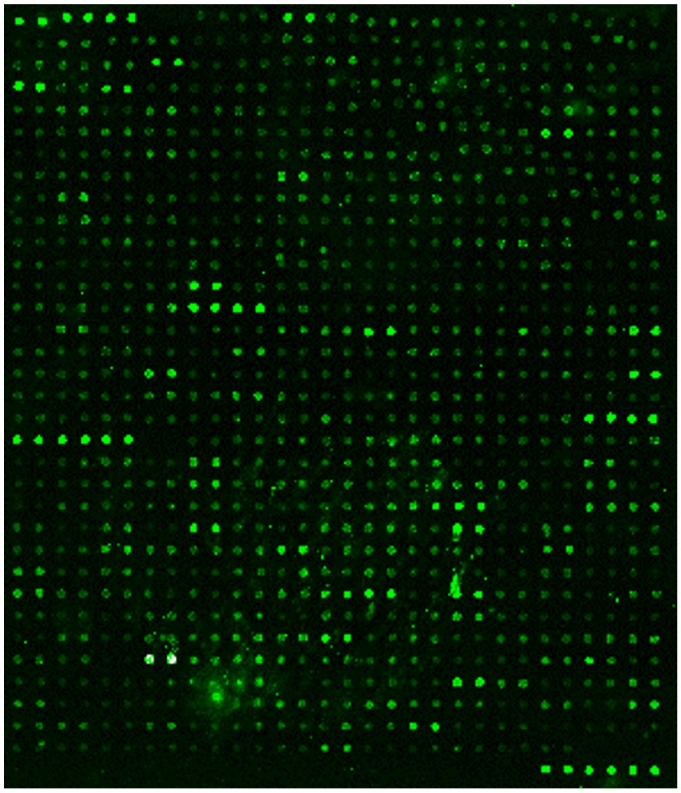
A. Explanation of Controls Spots

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.

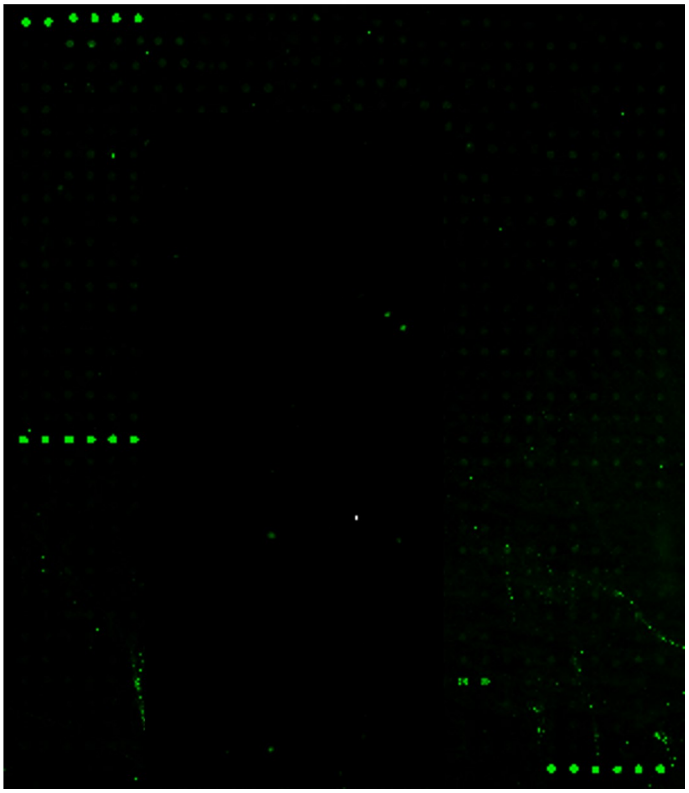
B. Typical Results

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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