RayBio® Label-Based (L-Series) Human L8 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-8-4 (4 Sample Kit)
AAH-BLG-8-8 (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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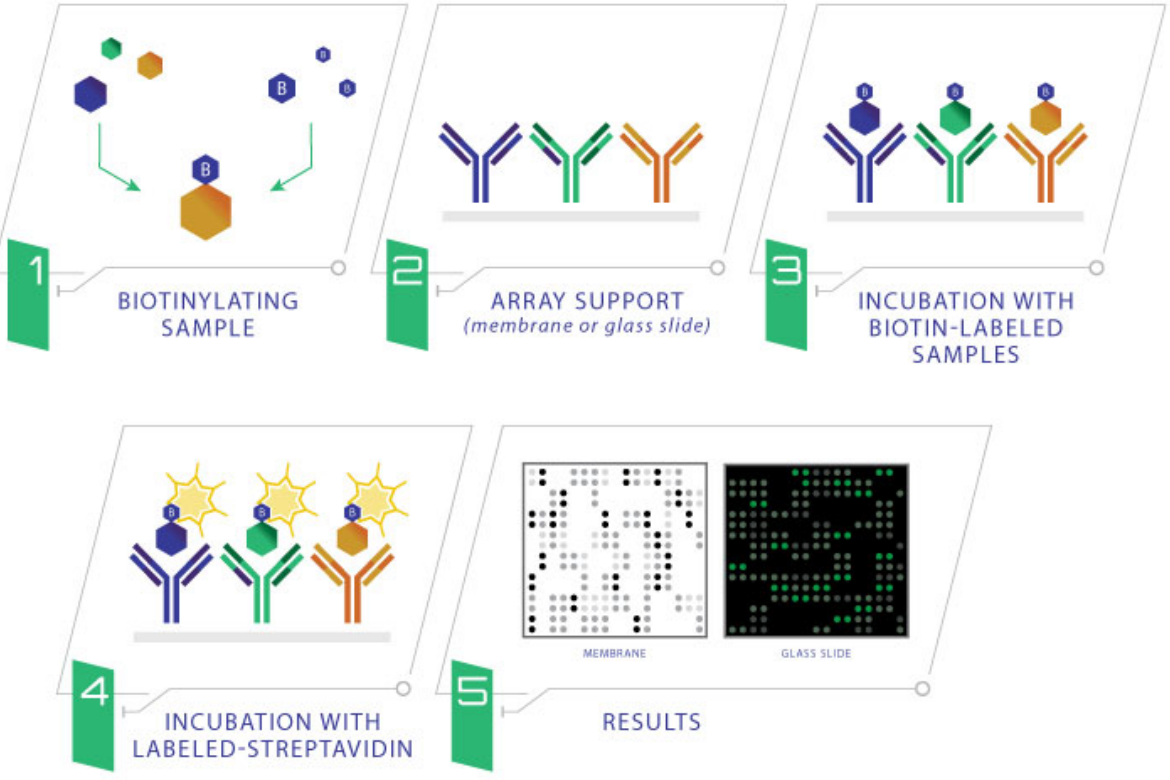
I. Introduction

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.

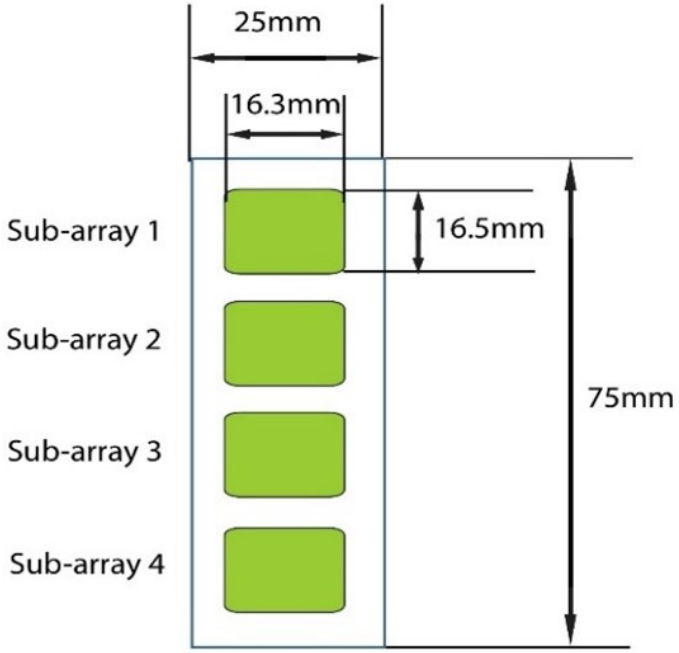
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

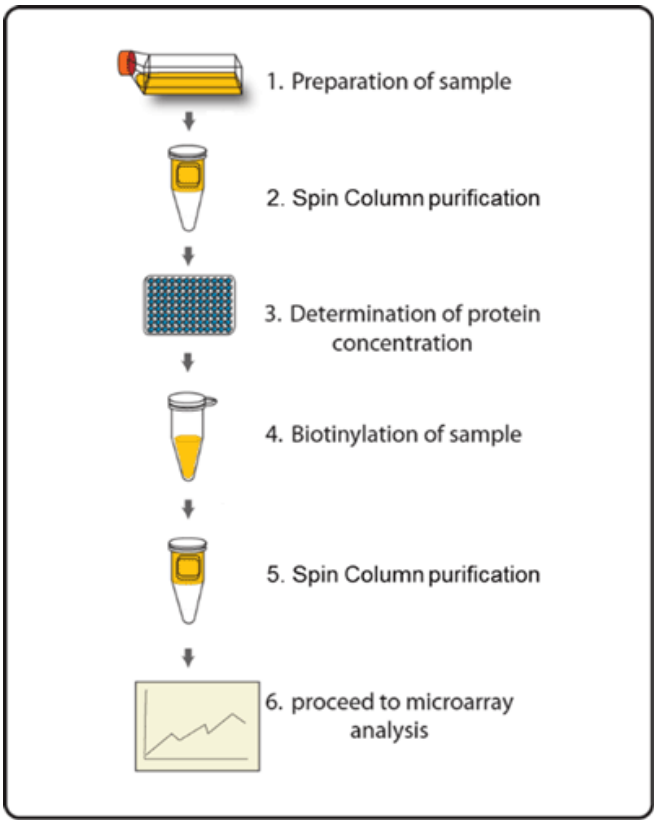
D. Incubations and Washes

- 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.

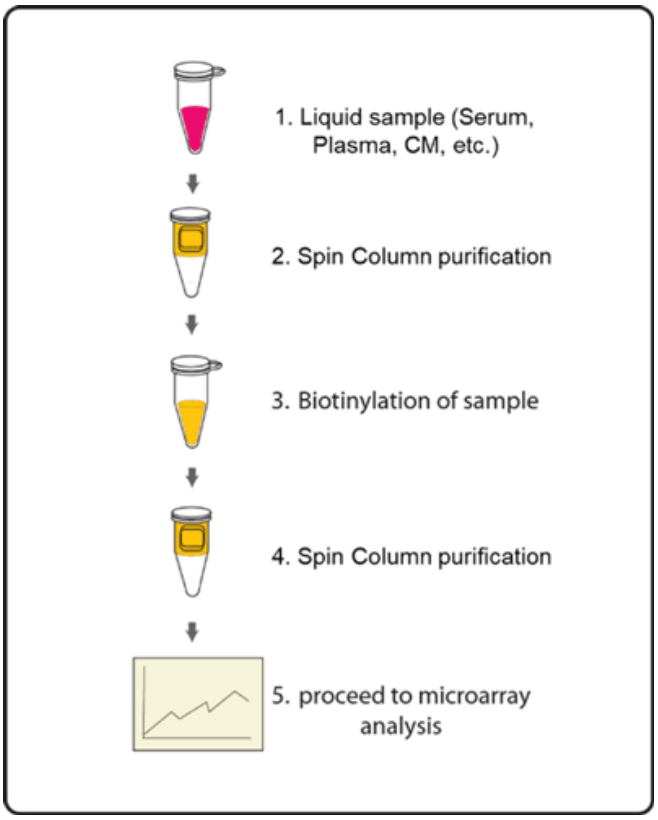
IV. Protocol

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.

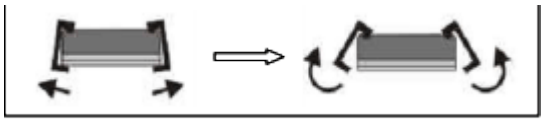
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

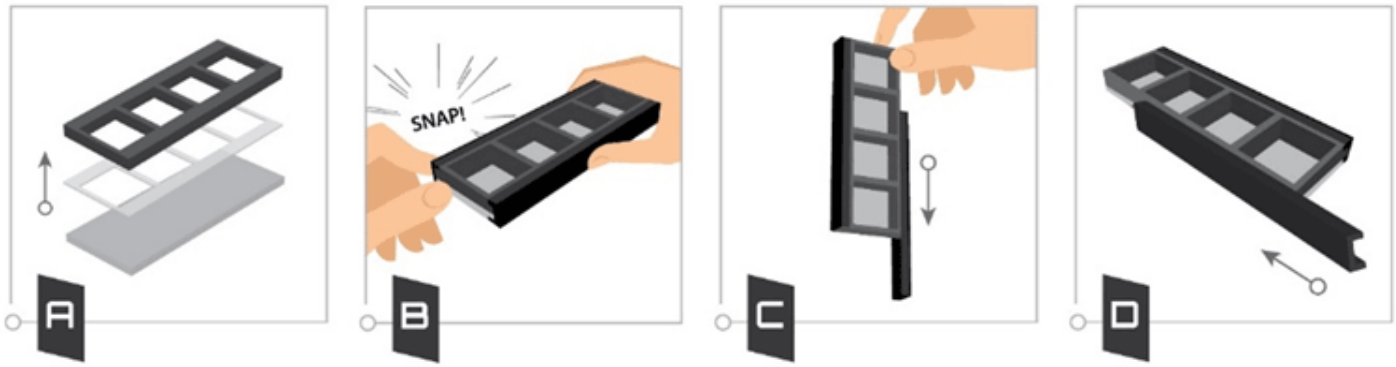
E. Fluorescence Detection

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	AASDHPPT	73	HPS3	145	MSC	217	PLP2	289	SCFD1	361	TCAP	433	WBSCR22
2	ABHD12B	74	HRASLS2	146	MX2	218	PNKP	290	SCN3B	362	TCEAL3	434	WDR4
3	ACADS	75	HSDL2	147	MYH11	219	PNMA2	291	SCO1	363	TCTN3	435	WDR44
4	ACADSB	76	HSF2BP	148	MYO10	220	POGK	292	SCRT2	364	TEP1	436	WDR54
5	ACADVL	77	HSFY1	149	MYO9B	221	POLQ	293	SCYL1	365	TGM6	437	WDR64
6	AGXT2	78	HTATIP2	150	NAA50	222	POPDC2	294	SEC14L3	366	THYN1	438	WDR77
7	ALAS2	79	HTATSF1	151	NACC1	223	PPIL3	295	SELENON	367	TIMM8A	439	WNK3
8	ALDH18A1	80	IER5	152	NAP1L2	224	PPME1	296	SETBP1	368	TIPRL	440	ZADH2
9	ALDH1L1	81	IFIT1	153	NCAPH	225	PPP1R18	297	SFTPA1	369	TM9SF1	441	ZBTB12
10	ALDH3A2	82	IFIT2	154	NDE1	226	PPP1R7	298	SH3BGR	370	TM9SF3	442	ZBTB2
11	ALDH5A1	83	IGHG4	155	NDRG3	227	PPP2R1A	299	SHROOM2	371	TMCC3	443	ZBTB26
12	ALDH8A1	84	IPP	156	NDRG4	228	PPP2R2A	300	SKAP2	372	TMED2	444	ZBTB3
13	AMDHD1	85	KCNH2	157	NECAP2	229	PPP4R2	301	SLA2	373	TMEM109	445	ZBTB45
14	ANKRD9	86	KCNH3	158	NEK3	230	PRMT7	302	SLC13A2	374	TMEM115	446	ZBTB46
15	AQP12A	87	KCNH6	159	NFKBIL1	231	PROSC	303	SLC13A3	375	TMEM132A	447	ZBTB9
16	ARF1	88	KCNK12	160	NFYA	232	PRPSAP2	304	SLC16A8	376	TMEM184B	448	ZC3H3
17	ARG2	89	KCNK13	161	NIFK	233	PRRC1	305	SLC1A4	377	TMEM260	449	ZDHHC11
18	ARHGEF25	90	KCTD13	162	NIN	234	PRRG1	306	SLC25A14	378	TMEM43	450	ZDHHC13
19	ARHGEF26	91	KCTD4	163	NKX3-2	235	PRSS16	307	SLC25A19	379	TMEM59L	451	ZDHHC14
20	ARPC1A	92	KDM2A	164	NLE1	236	PRSS21	308	SLC25A22	380	TMEM9	452	ZFAND1
21	ASB3	93	KIAA0825	165	NLRC4	237	PSG4	309	SLC25A29	381	TMEM91	453	ZFP28
22	ASPA	94	KIAA1755	166	NOLC1	238	PSMG1	310	SLC25A32	382	TMOD2	454	ZFP64
23	ASPN	95	KIAA1958	167	NOP9	239	PSMG3	311	SLC25A39	383	TMPRSS4	455	ZFP91
24	ATP10D	96	KLHL12	168	NOVA1	240	PTGR2	312	SLC26A5	384	TNRC6B	456	ZFYVE19
25	ATP11C	97	KLHL40	169	NRIP3	241	QTRT1	313	SLC2A6	385	TOMM70A	457	ZFYVE28
26	BCL6B	98	KLHL41	170	NSMCE1	242	RAB15	314	SLC35C1	386	TOR1AIP2	458	ZIC5
27	BEST4	99	KLHL42	171	NTAN1	243	RAB3B	315	SLC35F6	387	TOX2	459	ZIM3
28	BRD4	100	KLHL5	172	NUDCD2	244	RAB5B	316	SLC37A1	388	TP53RK	460	ZKSCAN1
29	C2CD2L	101	KRT23	173	NUDT11	245	RAB5C	317	SLC39A2	389	TPD52L2	461	ZMYM2
30	CALCOCO2	102	KRT6A	174	NUTF2	246	RAB6C	318	SLC39A7	390	TRAF3IP3	462	ZMYM3
31	CASP4	103	KRT6C	175	NXF1	247	RABEP2	319	SLC45A4	391	TRAPPC2L	463	ZMYM5
32	CDC27	104	KRT76	176	OGFR	248	RABGAP1	320	SLC4A10	392	TRAPPC6B	464	ZMYM6
33	CDH20	105	LCN15	177	OLFML1	249	RABGGTA	321	SLC7A3	393	TRIM29	465	ZNF100
34	CDH24	106	LEPREL1	178	OPN1MW	250	RALY	322	SLCO5A1	394	TRIM3	466	ZNF101
35	CDH26	107	LHX2	179	OSBPL9	251	RALYL	323	SLMAP	395	TRIM33	467	ZNF117
36	CDH9	108	LIMCH1	180	OSER1	252	RANBP3	324	SMARCA2	396	TRIM34	468	ZNF140
37	CENPB	109	LIN7A	181	OSTF1	253	RANBP9	325	SMARCC1	397	TRIM56	469	ZNF195
38	CEP41	110	LIN7C	182	OTUD6B	254	RBCK1	326	SMARCD1	398	TRIO	470	ZNF205
39	CLCN3	111	LMCD1	183	OVGP1	255	RBM11	327	SNAPC5	399	TRIP13	471	ZNF248
40	CLCN5	112	LPXN	184	OXER1	256	RBM14	328	SNX10	400	TRPM3	472	ZNF250
41	CLCN7	113	LRRC14	185	OXR1	257	RBM3	329	SNX15	401	TSC22D2	473	ZNF264
42	CLDN18	114	LRRC2	186	P4HTM	258	RBM45	330	SNX9	402	TSNAX	474	ZNF275
43	CLMN	115	LRRC25	187	PACSIN1	259	RBM7	331	SON	403	TSPAN10	475	ZNF285
44	CTIF	116	LRRC59	188	PACSIN2	260	RBP1	332	SOX12	404	TSPAN3	476	ZNF300
45	DHX34	117	LUC7L2	189	PACSIN3	261	RCN1	333	SP140	405	TSPAN31	477	ZNF319
46	DHX35	118	LYG2	190	PADI3	262	RECQL	334	SPAG11B	406	TSPAN5	478	ZNF320
47	DHX37	119	LYPLAL1	191	PAGE1	263	RFPL3	335	SPC24	407	TSSC1	479	ZNF335
48	DIP2A	120	LZTFL1	192	PAPLN	264	RFX2	336	SPPL2B	408	TSSC4	480	ZNF33A
49	DMTN	121	MAK10	193	PCDHB16	265	RGS22	337	SPPL3	409	TSTD1	481	ZNF354C
50	DYNC1LI1	122	MAP1B	194	PCDHB2	266	RHAG	338	SRRM2	410	TTC12	482	ZNF407
51	DYSF	123	MAP3K7CL	195	PCDHB5	267	RHBDL2	339	SSRP1	411	TTC17	483	ZNF451
52	EEF1A2	124	MAP7	196	PCM1	268	RHOF	340	ST6GALNAC2	412	TTC19	484	ZNF462
53	EVI2B	125	MAP7D2	197	PCMTD1	269	RHOG	341	STAU1	413	TTLL12	485	ZNF501
54	EXOG	126	MAPRE2	198	PCYOX1L	270	RIC8A	342	STOML1	414	TUFM	486	ZNF502
55	FAM71D	127	MARK3	199	PDAP1	271	RNF114	343	STOML2	415	TWSG1	487	ZNF512
56	FBXL5	128	MAST4	200	PDE4D	272	RNF146	344	SUPT16H	416	TXNDC16	488	ZNF543
57	GABRD	129	MBNL2	201	PDIA2	273	RNF25	345	SUPT5H	417	TXNL1	489	ZNF558
58	GLIS3	130	MBTD1	202	PDIA5	274	RNF38	346	SUSD6	418	UBAP2L	490	ZNF580
59	GPS2	131	MED8	203	PDLIM4	275	RNPEPL1	347	SYNE1	419	UBLCP1	491	ZNF599
60	GRIP1	132	MFAP3L	204	PDPR	276	RPAP3	348	SYNGR1	420	UBTF	492	ZNF606
61	GSC2	133	MIS12	205	PDS5A	277	RRAGB	349	SYT4	421	UBXN1	493	ZNF644
62	HCFC2	134	MOB4	206	PDXDC1	278	RSL1D1	350	SYT5	422	UBXN10	494	ZNF665
63	HDDC2	135	MORC3	207	PGRMC1	279	RUNDC3A	351	TADA2A	423	UBXN4	495	ZNF684
64	HDGFRP2	136	MOXD1	208	PHF21A	280	RWDD4	352	TAPT1	424	UCHL5	496	ZNF799
65	HDHD2	137	MPZL2	209	PHF6	281	SAFB2	353	TATDN1	425	UEVLD	497	ZNF823
66	HDHD3	138	MRGPRX3	210	PHGR1	282	SAMM50	354	TBC1D10B	426	USP10	498	ZNF93
67	HDLBP	139	MRPL37	211	PHLDB2	283	SAR1A	355	TBC1D2	427	USP18	499	ZNHIT3
68	HEBP1	140	MRPL40	212	PIN4	284	SATB2	356	TBC1D9B	428	USP26	500	ZW10
69	HLA-H	141	MRPS22	213	PITPNB	285	SBNO1	357	TBCC	429	VGLL2		
70	HMCN1	142	MRPS23	214	PITRM1	286	SCAF8	358	TBCE	430	VPS16		
71	HMG20A	143	MRPS26	215	PLEKHF2	287	SCAMP5	359	TBRG4	431	VPS53		
72	HOMEZ	144	MRPS36	216	PLLP	288	SCAPER	360	TBX10	432	VSTM2L		

VII. Interpretation of Results:

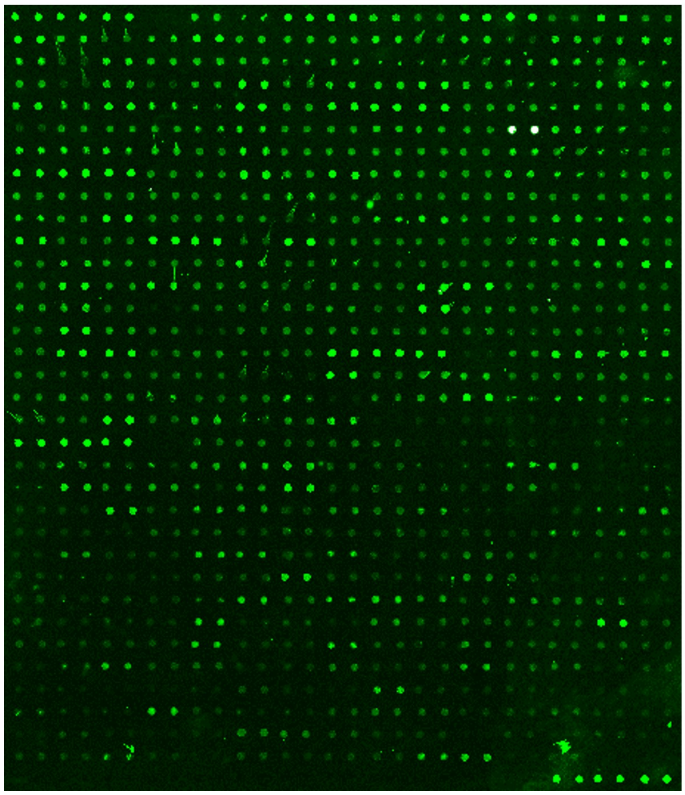
A. Explanation of Controls Spots

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.

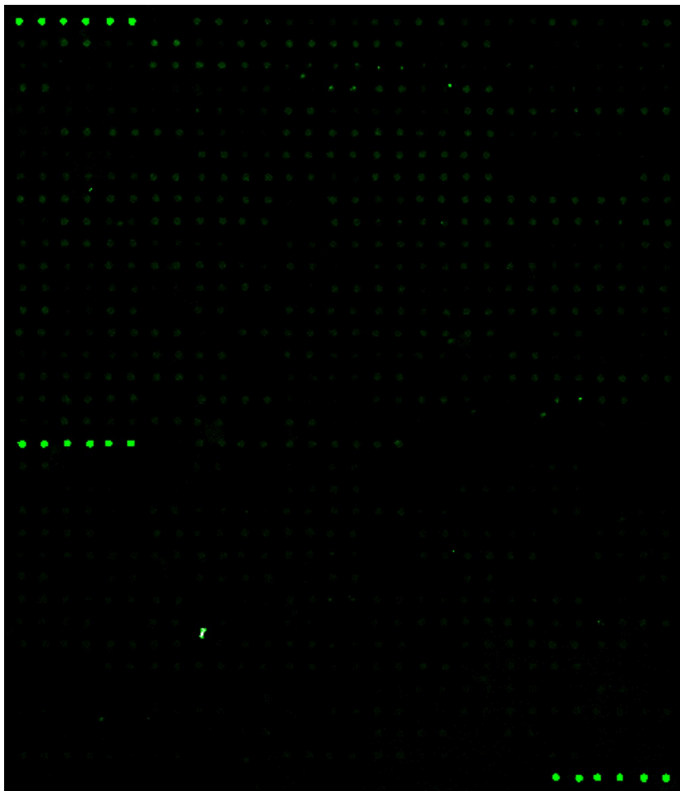
B. Typical Results

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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