RayBio® Label-Based (L-Series) Human Infectious disease screening array

Patent Pending Technology
User Manual (Apr 14, 2023)

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-IFD-4 (4 Sample Kit)
AAH-BLG-IFD-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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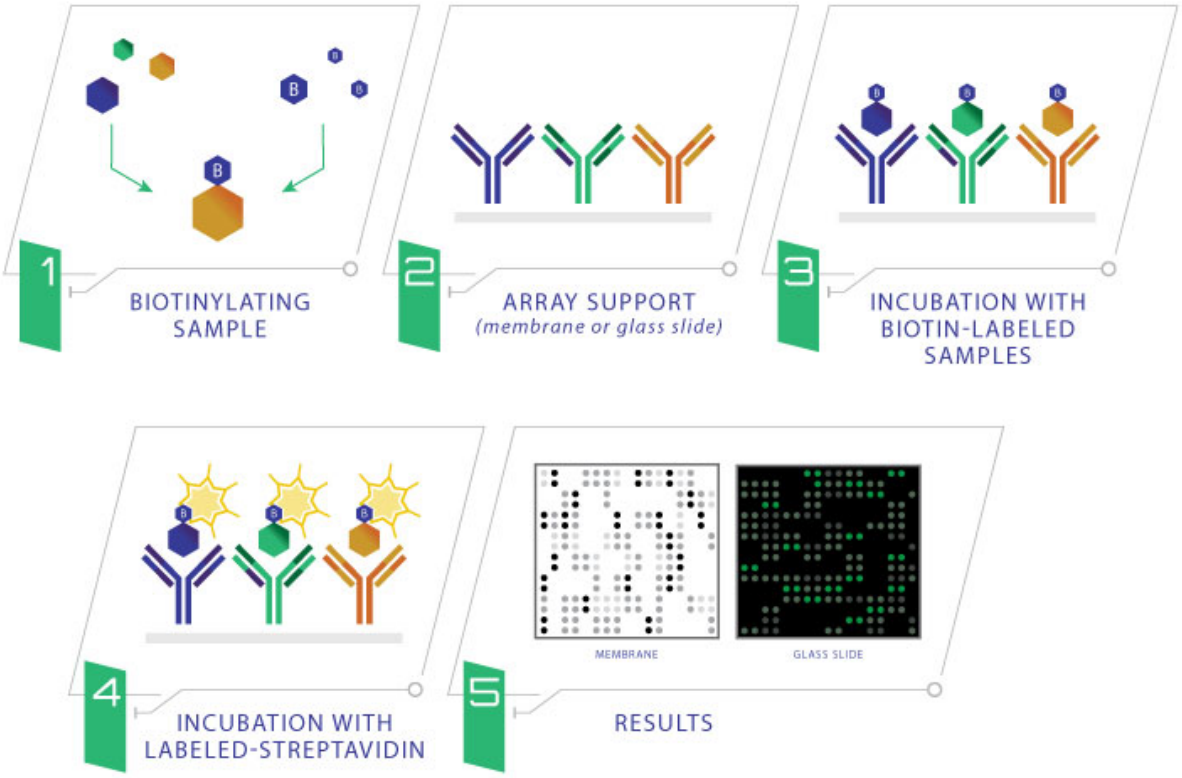
I. Introduction

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

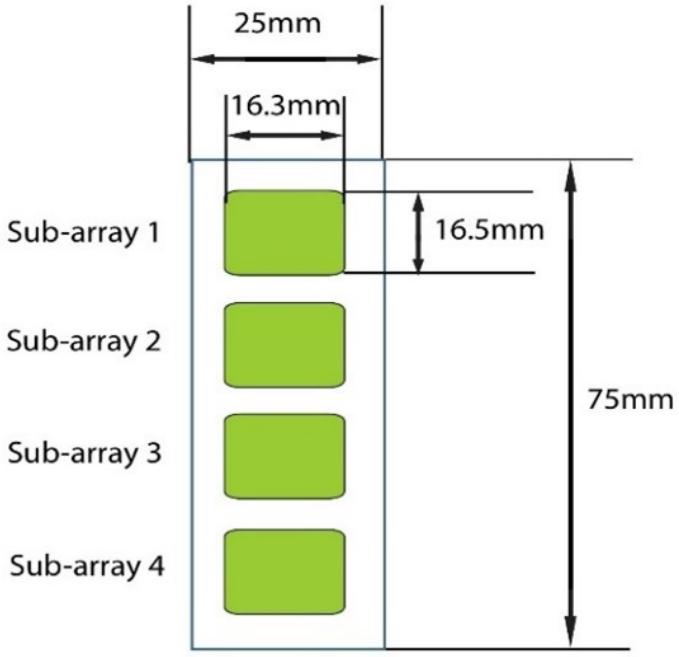
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

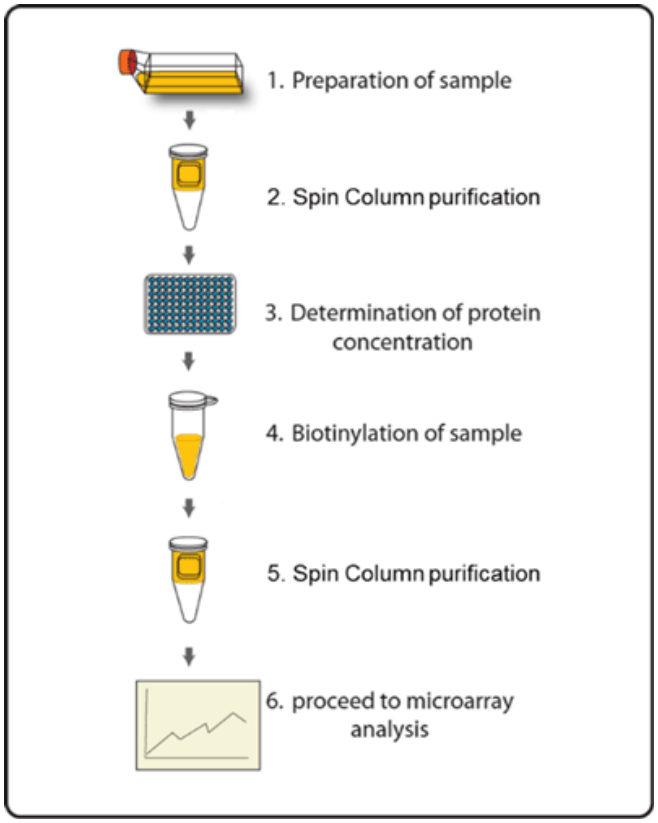
D. Incubations and Washes

- 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.

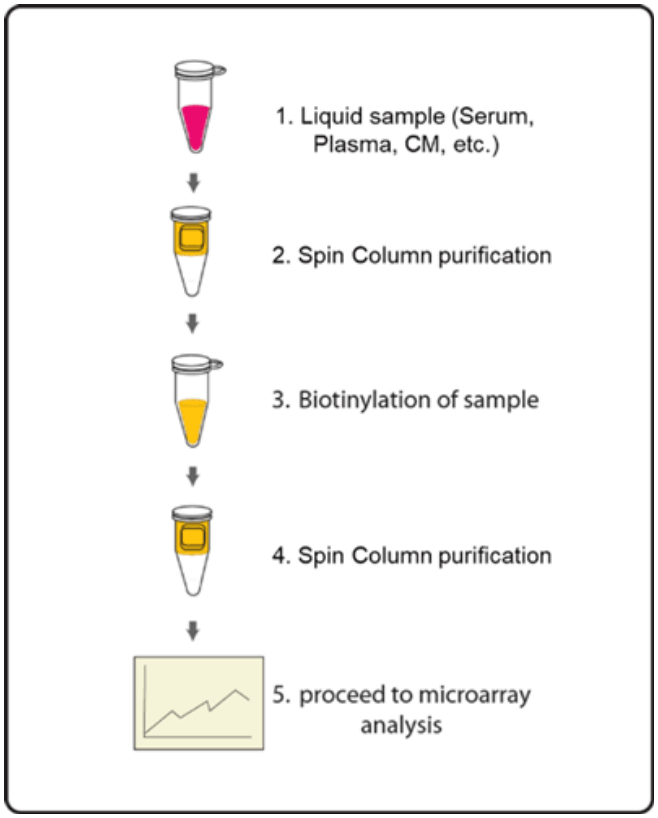
IV. Protocol

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 not 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 completely 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.

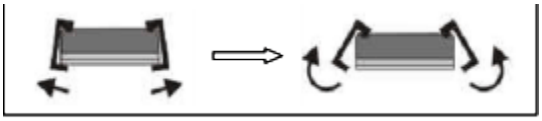
    Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

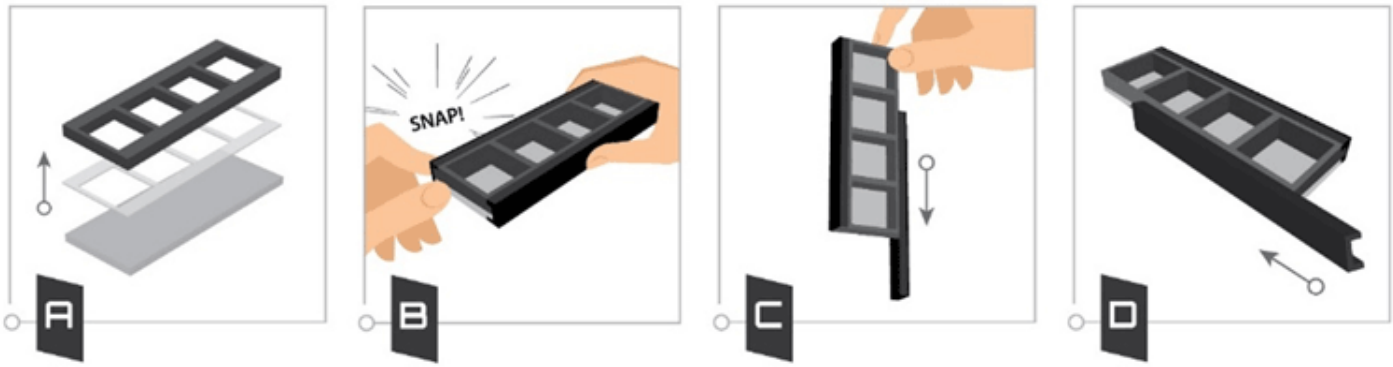
E. Fluorescence Detection

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: Please protect the finished glass slides from temperatures above RT and store them in the dark. 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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1	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11
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VI. Antibody Array Target List

Infectious disease target list

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	ABI1	73	CALM2	145	CREB3L2	217	HLA-DQA2	289	LAMB2	361	PIK3CA	433	SMAD2
2	ACTN1	74	CALML3	146	CREBBP	218	HLA-DRB1	290	LDLR	362	PIK3CB	434	SMAD3
3	ADAR	75	CALR	147	CRK	219	HLA-DRB3	291	LTBR	363	PIK3CD	435	SMAD4
4	ADCY1	76	CARD9	148	CRKL	220	HLA-E	292	LY96	364	PIK3CG	436	SOCS3
5	ADCY3	77	CASP1	149	CSF2	221	HRAS	293	MAD1L1	365	PIK3R1	437	SOS2
6	ADCY9	78	CASP10	150	CTNNB1	222	HSPA1A	294	MAD2L1	366	PIK3R2	438	SRC
7	AKT1	79	CASP3	151	CTTN	223	HSPA1L	295	MALT1	367	PIK3R3	439	STAT1
8	AKT2	80	CASP4	152	CXCL1	224	HSPA2	296	MAP2K1	368	PIK3R5	440	STAT2
9	AKT3	81	CASP7	153	CXCL10	225	HSPA6	297	MAP2K2	369	PIK3R6	441	STAT3
10	ANGPT2	82	CASP8	154	CXCL2	226	HSPA8	298	MAP2K3	370	PLCB1	442	STAT4
11	APAF1	83	CASP9	155	CXCL3	227	HSPD1	299	MAP2K4	371	PLCB2	443	STAT5A
12	ARAF	84	CCL2	156	CYCS	228	HSPG2	300	MAP2K7	372	PLCB3	444	STAT5B
13	ARF1	85	CCL5	157	CYFIP2	229	ICAM1	301	MAP3K1	373	PLCB4	445	STAT6
14	ARF6	86	CCNA1	158	CYTH1	230	IFNA10	302	MAP3K14	374	PLCG1	446	SYK
15	ARHGEF12	87	CCNA2	159	CYTH2	231	IFNA14	303	MAP3K3	375	PLCG2	447	TAP1
16	ARHGEF2	88	CCND1	160	CYTH3	232	IFNA16	304	MAP3K7	376	PLG	448	TAP2
17	ARPC1A	89	CCND2	161	CYTH4	233	IFNA17	305	MAP3K7IP1	377	PML	449	TAPBP
18	ARPC1B	90	CCND3	162	DDB2	234	IFNA2	306	MAPK1	378	PPARA	450	TBK1
19	ARPC2	91	CCNE1	163	DDX58	235	IFNA21	307	MAPK11	379	PPP1CA	451	TCIRG1
20	ARPC3	92	CCNE2	164	DNAJB1	236	IFNA4	308	MAPK12	380	PPP1CC	452	TGFB1
21	ARPC4	93	CCR1	165	DNM2	237	IFNA5	309	MAPK13	381	PPP2CA	453	TGFB2
22	ARPC5	94	CCR3	166	DOCK1	238	IFNA6	310	MAPK14	382	PPP2CB	454	TGFB3
23	ATF2	95	CCR4	167	E2F1	239	IFNA7	311	MAPK3	383	PPP2R1A	455	TGFBR1
24	ATF4	96	CCR5	168	E2F2	240	IFNA8	312	MAPK8	384	PPP2R2A	456	TGFBR2
25	ATG3	97	CCR8	169	EEF1A2	241	IFNAR1	313	MAPK9	385	PPP2R2B	457	TICAM2
26	ATM	98	CD14	170	EGF	242	IFNAR2	314	MDM2	386	PPP2R2C	458	TJP1
27	ATP6AP1	99	CD19	171	EGFR	243	IFNB1	315	MET	387	PPP3CA	459	TLN1
28	ATP6V0A1	100	CD200R1	172	EIF2AK2	244	IFNG	316	MICB	388	PPP3CB	460	TLN2
29	ATP6V0A2	101	CD209	173	EIF2AK3	245	IFNGR1	317	MMP7	389	PPP3R1	461	TLR2
30	ATP6V0A4	102	CD247	174	EIF2AK4	246	IFNGR2	318	MMP9	390	PPP3R2	462	TLR3
31	ATP6V0C	103	CD28	175	EIF2B3	247	IKBKB	319	MX1	391	PREX1	463	TLR4
32	ATP6V0D1	104	CD3D	176	EIF2S1	248	IKBKE	320	MYC	392	PRKACA	464	TLR5
33	ATP6V0E2	105	CD3E	177	EIF4EBP1	249	IKBKG	321	MYD88	393	PRKACB	465	TLR6
34	ATP6V1A	106	CD3G	178	ELK1	250	IL10	322	MYH11	394	PRKACG	466	TLR9
35	ATP6V1B1	107	CD4	179	ELMO1	251	IL10RA	323	MYH14	395	PRKCA	467	TNFAIP3
36	ATP6V1B2	108	CD40	180	EP300	252	IL10RB	324	MYH15	396	PRKCB	468	TNFRSF10A
37	ATP6V1C1	109	CD44	181	ETS1	253	IL12A	325	MYH4	397	PRKCG	469	TNFRSF10B
38	ATP6V1C2	110	CD58	182	FADD	254	IL12B	326	MYH8	398	PRSS1	470	TNFRSF14
39	ATP6V1D	111	CD81	183	FAS	255	IL15	327	MYL12A	399	PRSS2	471	TNFRSF1A
40	ATP6V1E1	112	CD86	184	FASLG	256	IL15RA	328	MYO6	400	PTGS2	472	TNFSF10
41	ATP6V1E2	113	CDC42	185	FCER2	257	IL18	329	NAIP	401	PTK2	473	TP53
42	ATP6V1F	114	CDK2	186	FCGR1A	258	IL1A	330	NCKAP1	402	PTPN11	474	TRADD
43	ATP6V1G1	115	CDK4	187	FCGR2A	259	IL1B	331	NCKAP1L	403	PTPN6	475	TRAF2
44	ATP6V1G2	116	CDK6	188	FCGR2B	260	IL1R1	332	NEDD4	404	PXN	476	TRAF3
45	ATP6V1G3	117	CDKN1A	189	FCGR2C	261	IL1R2	333	NFATC1	405	PYCARD	477	TRAF5
46	ATP6V1H	118	CDKN1B	190	FCGR3A	262	IL2	334	NFATC2	406	RAB1A	478	TRAF6
47	ATR	119	CDKN2A	191	FCGR3B	263	IL23A	335	NFATC3	407	RAB5A	479	TSC1
48	B2M	120	CDKN2B	192	FGF2	264	IL2RA	336	NFATC4	408	RAB5B	480	TSC2
49	BAD	121	CDKN2C	193	FN1	265	IL2RB	337	NFKB1	409	RAB5C	481	TYK2
50	BAK1	122	CFLAR	194	FOS	266	IL2RG	338	NFKB2	410	RAB7A	482	UBA52
51	BAX	123	CHUK	195	GABARAP	267	IL33	339	NFKBIA	411	RAB9B	483	UBB
52	BCAR1	124	CIITA	196	GABARAPL1	268	IL6	340	NFKBIB	412	RAC1	484	UBC
53	BCL10	125	CLDN1	197	GABARAPL2	269	IL6ST	341	NFKBIE	413	RAF1	485	VCAM1
54	BCL2	126	CLDN10	198	GNA14	270	ILK	342	NFYB	414	RB1	486	VCL
55	BCL2L1	127	CLDN11	199	GNA15	271	IRAK1	343	NLRC4	415	RELA	487	VDAC1
56	BCL2L11	128	CLDN14	200	GNAI1	272	IRAK4	344	NLRP3	416	RELB	488	VEGFA
57	BECN1	129	CLDN15	201	GNAI2	273	IRF3	345	NOD1	417	RHOA	489	VISA
58	BID	130	CLDN17	202	GNAI3	274	ITGA5	346	NOS2	418	RHOG	490	WASF1
59	BIRC2	131	CLDN18	203	GNAL	275	ITGAL	347	NRAS	419	RIPK1	491	WASF3
60	BIRC3	132	CLDN19	204	GNAO1	276	ITGAM	348	NRP1	420	RIPK2	492	WASL
61	BNIP3	133	CLDN2	205	GRB2	277	ITGB1	349	NXF1	421	RNASEL	493	XIAP
62	BRAF	134	CLDN23	206	GSK3B	278	ITGB2	350	NXF3	422	ROCK1	494	XPO1
63	BTK	135	CLDN3	207	HCK	279	ITGB3	351	NXF5	423	ROCK2	495	YWHAB
64	C1QA	136	CLDN4	208	HCLS1	280	JAK1	352	OAS1	424	RPS27A	496	YWHAE
65	C1QC	137	CLDN6	209	HIF1A	281	JAK3	353	OAS2	425	RSAD2	497	YWHAH
66	C1R	138	CLDN7	210	HLA-C	282	JUN	354	PAK1	426	RUNX3	498	YWHAQ
67	C1S	139	CLDN8	211	HLA-DMA	283	KNG1	355	PAK3	427	SELE	499	YWHAZ
68	C2	140	CLDN9	212	HLA-DMB	284	KPNA1	356	PDIA3	428	SELP	500	ZFP36
69	C3	141	CLEC4M	213	HLA-DOA	285	KRAS	357	PFN1	429	SLC25A31		
70	C4A	142	CR1	214	HLA-DOB	286	LAMA1	358	PFN2	430	SLC25A4		
71	C5	143	CREB1	215	HLA-DPA1	287	LAMA3	359	PFN4	431	SLC25A5		
72	CALCOCO2	144	CREB3L1	216	HLA-DQA1	288	LAMA4	360	PIK3C3	432	SLC25A6		

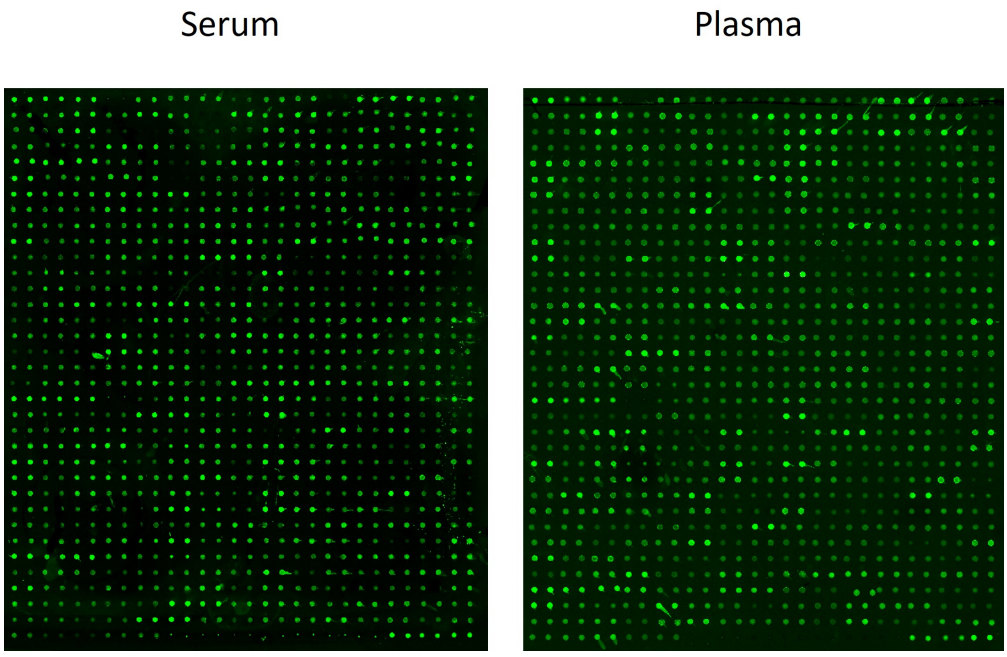
VII. Interpretation of Results

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.



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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