RayBio® Label-Based (L-Series) Human L16 Array, Glass Slide

Patent Pending Technology
User Manual (Oct 13, 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-16-4 (4 Sample Kit)
AAH-BLG-16-8 (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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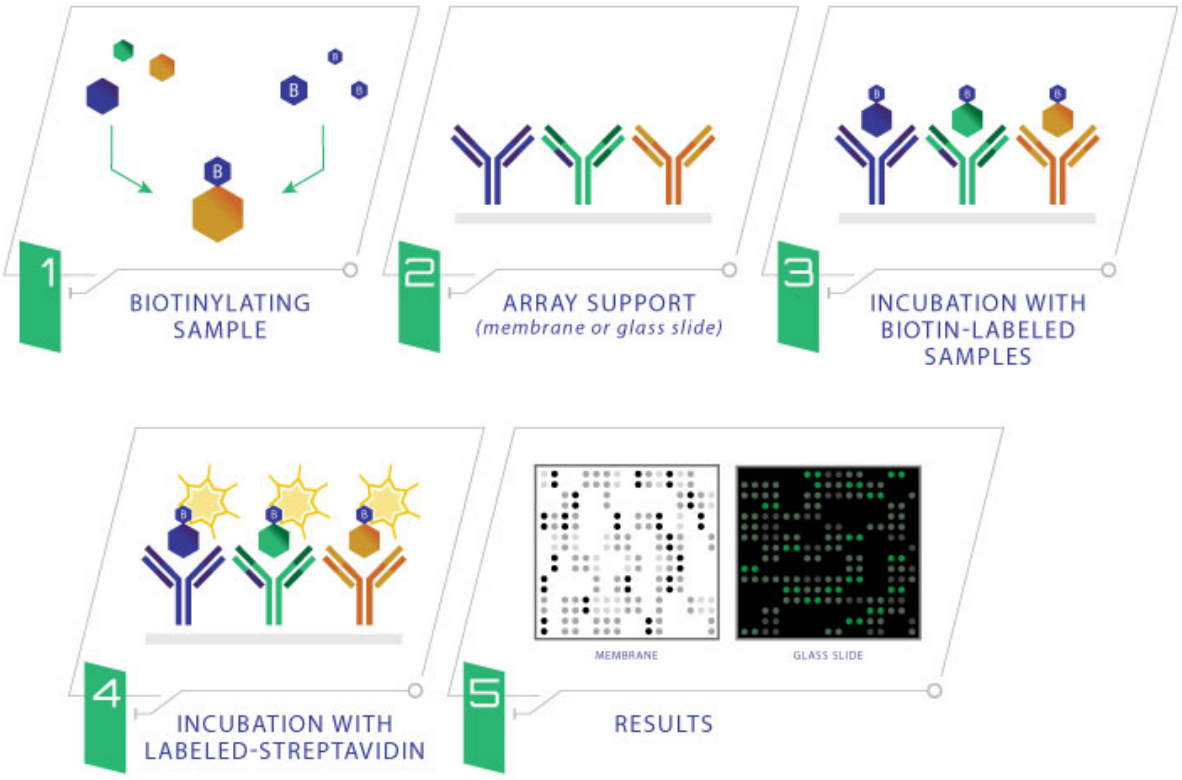
I. Introduction

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.

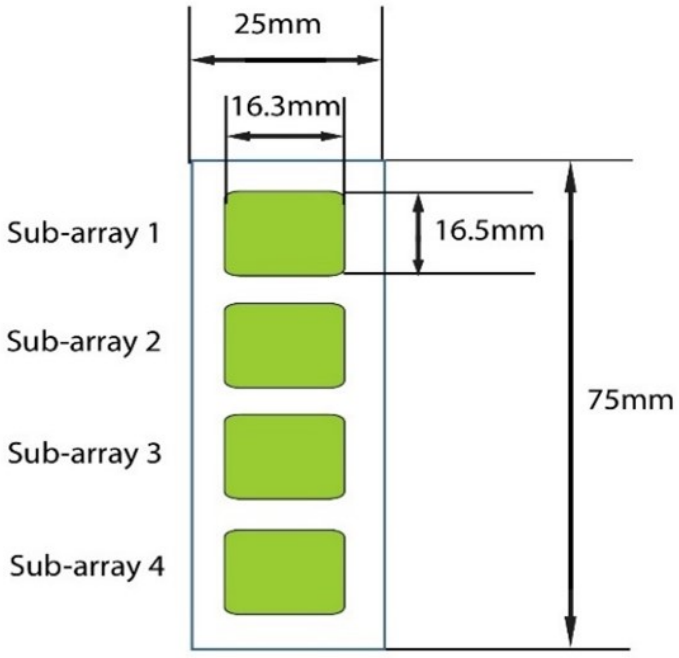
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

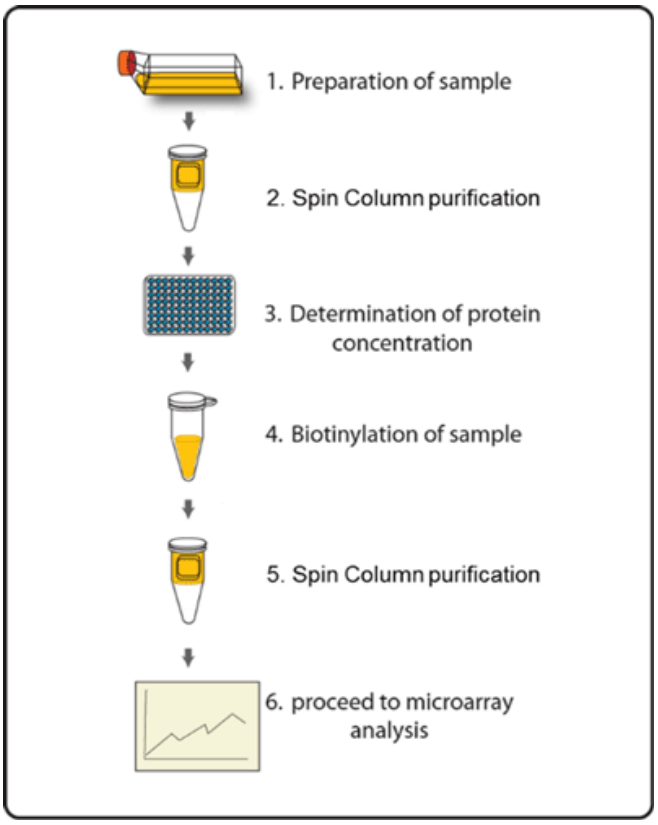
D. Incubations and Washes

- 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.

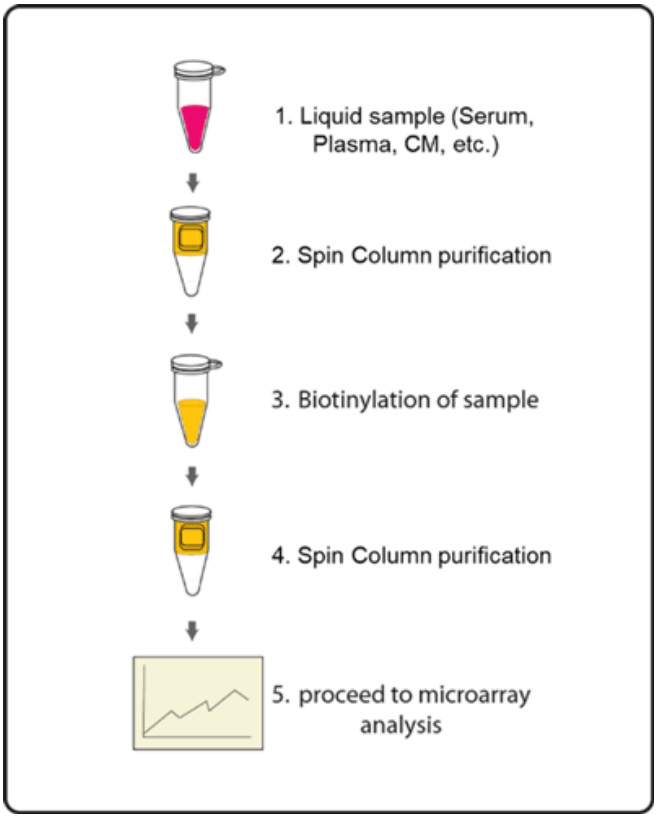
IV. Protocol

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 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.

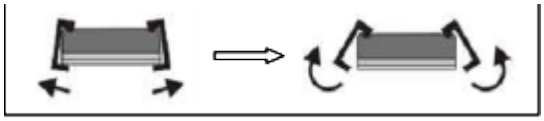
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

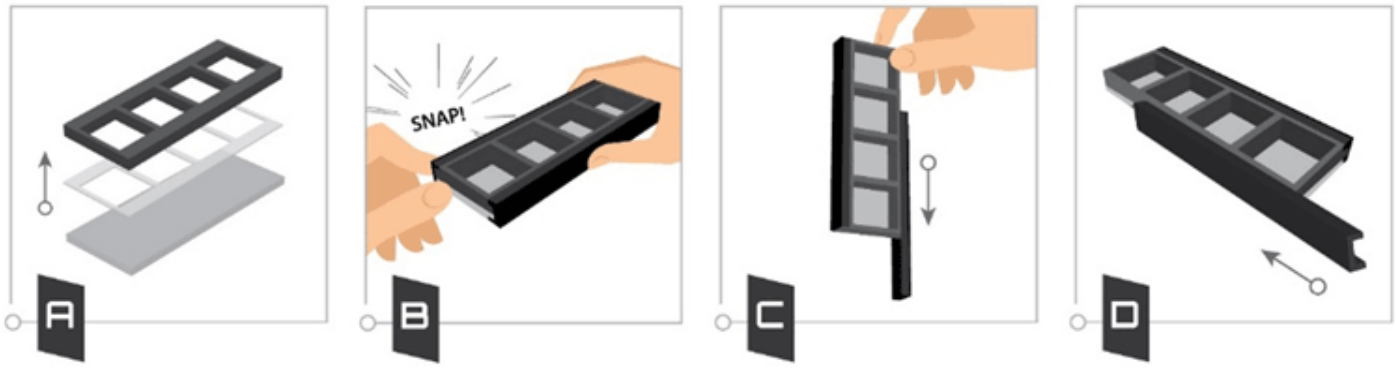
E. Fluorescence Detection

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
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2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
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35	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	0S Proteasom	73	CCL4L1	145	EIF4EBP3	217	HOMER2	289	MED27	361	PGK2	433	SIRT6
2	A2ML1	74	CCN6	146	ELF3	218	HOXB7	290	MED7	362	PGP	434	SIX4
3	AACS	75	CCNB2	147	ELK1	219	HSCB	291	MEGF10	363	PKP4	435	SKA1
4	AADAT	76	CD160	148	EMX2	220	HSD17B11	292	METRNL	364	PLA2G10	436	SLAIN1
5	AAGAB	77	CD164L2	149	EPB41L1	221	HSD17B14	293	MFAP3	365	PLA2G2D	437	SLAMF9
6	AATK	78	CD300LD	150	EPGN	222	HSP90AA1	294	MINDY2	366	PLAC8	438	SLURP2
7	ABHD10	79	CD3G	151	ERP27	223	HSPB3	295	MLN	367	PLCXD3	439	SMOC1
8	ABHD4	80	CD68	152	EXOSC7	224	HSPB9	296	MMGT1	368	PLK1	440	SMR3B
9	ABRACL	81	CD96	153	FABP12	225	HYAL4	297	MMP26	369	PLPPR4	441	SNRNP25
10	ACBD6	82	CDK2AP2	154	FABP9	226	HYKK	298	MOB3B	370	PLXDC1	442	SNRPB2
11	ACBD7	83	CDKN2AIPNL	155	FAHD1	227	IDH3G	299	MOSPD1	371	PNLIPRP2	443	SORBS3
12	ACOT1	84	CEACAM18	156	FAIM3	228	IDNK	300	MPZL3	372	PNMT	444	SPAG16
13	ACRV1	85	CEBPG	157	FAM107B	229	IER3	301	MREG	373	POLD4	445	SPECC1
14	ACTA1	86	CEP20	158	FAM171B	230	IFNA14	302	MRFAP1L1	374	POMZP3	446	SPRR1B
15	ADAL	87	CEP43	159	FAT10	231	IFNA16	303	MRM1	375	POU5F1B	447	SPRY3
16	ADAT1	88	CETN1	160	FBLN7	232	IFNA21	304	MRM3	376	POU6F1	448	SPRY4
17	ADGRL3	89	CFAP36	161	FCRLA	233	IFT20	305	MSRA	377	PPM1A	449	SSR2
18	AHSA1	90	CGB7	162	FDCSP	234	IFT22	306	MSRB3	378	PPP3CA	450	SSU72
19	AIDA	91	CIDEA	163	FGF1	235	IGFL2	307	MST4	379	PRH1	451	ST6GAL2
20	AK4	92	CINP	164	FIBP	236	IGFL3	308	MTCP1	380	PRKCQ	452	STARD5
21	ALDH1A3	93	CLDN11	165	FKBPL	237	IGFLR1	309	MXD3	381	PRKCZ	453	STAT4
22	AMELX	94	CLEC12B	166	FLIP	238	IGHE	310	MYCL	382	PRKRA	454	STIM1
23	AMMECR1L	95	CLEC17A	167	FREM1	239	IGKC	311	MYL12A	383	PRND	455	STN1
24	AMY1A	96	CLEC2A	168	FRMPD4	240	IGLON5	312	NACA	384	PROK2	456	SULT1A2
25	AP1AR	97	CLEC2B	169	FRS2	241	IGSF9B	313	NAIF1	385	PRRT2	457	SULT1A3
26	APMAP	98	CLEC2L	170	FUBI	242	IL-36 Ra	314	NDEL1	386	PTBP1	458	SULT1E1
27	ARHGEF39	99	CLSTN3	171	FUOM	243	ILDR2	315	NDST2	387	PTH2	459	SYNC
28	ARL14	100	CLTRN	172	FUT10	244	IMPACT	316	NDUFB4	388	PTPMT1	460	SYT13
29	ARL4D	101	CMC4	173	FUT2	245	IMPDH1	317	NEDD4	389	PTPRB	461	TBX21
30	ARL9	102	CNOT9	174	FUT9	246	INSL4	318	NEU2	390	RAB17	462	TCEA2
31	ARMS2	103	CNPY4	175	GAB3	247	IRGM	319	NFU1	391	RAB1A	463	TCEB1
32	ASB13	104	CNTNAP1	176	GAGE12F	248	IZUMO1	320	NHLH2	392	RAB34	464	TECTB
33	ASIP	105	COL13A1	177	GAGE2A	249	IZUMO4	321	NIPSNAP1	393	RABIF	465	TENM1
34	ASMT	106	COL23A1	178	GALNT7	250	JMJD6	322	NKIRAS1	394	RAC1	466	TFB2M
35	ASPHD1	107	COLGALT2	179	GATA-6	251	JMJD7	323	NMI	395	RAC2	467	TGFBRAP1
36	ATL1	108	COMMD7	180	GET4	252	JOSD1	324	NMNAT2	396	RAET1G	468	TGIF2LY
37	ATM	109	COQ9	181	GFRAL	253	JPT2	325	NOTCH2NL	397	RARRES3	469	THAP3
38	ATP5PD	110	COX1	182	GGACT	254	JSRP1	326	NPC2	398	RASSF3	470	TIPIN
39	ATXN3L	111	CPLANE2	183	GGT5	255	JTB	327	NPR2	399	RBFOX1	471	TLR10
40	B3GALT5	112	CPPED1	184	GKN2	256	KCT2	328	NR0B1	400	RCN3	472	TLR6
41	B3GNT6	113	CRIPT	185	GKN3P	257	KDM3A	329	NR1C2	401	RDH14	473	TM4SF4
42	B9D1	114	CRYGD	186	GLDN	258	KDM6B	330	NR2F2	402	REL	474	TMED9
43	BAALC	115	CRYGN	187	GLUL	259	KIAA0664	331	NR3C1	403	REV1	475	TMEM204
44	BAG3	116	CRYGS	188	GNE	260	KIRREL1	332	NR5A1	404	RGS1	476	TMIGD2
45	BCAP	117	CSDC2	189	GNG11	261	KLC2	333	NRN1L	405	RGS21	477	TMPRSS11B
46	BCDIN3D	118	CSMD3	190	GNRH2	262	KLRG1	334	NRXN1	406	RNASE7	478	TNNC2
47	BEST1	119	CSN2	191	GP2	263	KRT34	335	NRXN2	407	RND1	479	TPSAB1
48	BEX1	120	CSPG5	192	GPHA2	264	KRT81	336	NRXN3	408	RNF17	480	TREX2
49	BLOC1S2	121	CTSW	193	GPHB5	265	KXD1	337	NUDT10	409	RP9	481	TRIM62
50	BLOC1S6	122	CUEDC2	194	GPIHBP1	266	LAGE3	338	NUDT3	410	RPA2	482	TTC32
51	BOLA3	123	CUZD1	195	GPR133	267	LAMTOR2	339	NUDT4	411	RPL26	483	TTC33
52	BPIFA2	124	CYREN	196	GPR64	268	LAMTOR4	340	NUP62CL	412	RPL35A	484	UBE2Q1
53	BRDG1	125	CYTIP	197	GPRC5D	269	LBH	341	OAF	413	RPLP1	485	UBE2R2
54	BTLA	126	DBNDD2	198	GRIN1	270	LGALS14	342	OAZ1	414	RPRD1A	486	UBE2S
55	C1QBP	127	DCX	199	GRIN2A	271	LILRA5	343	OCM	415	RPS18	487	UBTD2
56	C1QL3	128	DEFA3	200	GRIN2C	272	LIN28B	344	OMP	416	RSPO4	488	UPRT
57	C1QL4	129	DEFA4	201	GSTM2	273	GP6	345	OSR2	417	S100A7A	489	UTF1
58	C1QTNF8	130	DEFB119	202	GSTT2	274	LMAN2L	346	OSTM1	418	S100G	490	VMO1
59	C4C	131	DERA	203	GTF3C6	275	LMX1B	347	OTUD3	419	S100Z	491	VSIG1
60	CADM2	132	DGAT2	204	GTSF1	276	LRRC3B	348	OVCA2	420	SAMD13	492	WDR48
61	CALCB	133	DNAJC24	205	GYPC	277	NATD1	349	PAGR1	421	SDHAF1	493	WFDC12
62	CAMK2A	134	DOC2A	206	HBG1	278	LY6D	350	PAMR1	422	SDHAF2	494	YES1
63	CAMK2N1	135	DPPA3	207	HBP1	279	LYPLA2	351	PANX3	423	SELENOF	495	YOD1
64	CAMK2N2	136	DTNBP1	208	HDAC2	280	LYZL2	352	PBLD	424	SELENOW	496	ZFP42
65	CAMKV	137	DUSP23	209	HDAC8	281	MAB21L2	353	PCDH10	425	SEMA3B	497	ZNF24
66	CASK	138	DYDC2	210	HDDC3	282	MAEA	354	PCID2	426	SEMA5B	498	ZNF514
67	CBL	139	DYNLRB1	211	HDGFL1	283	MAGOHB	355	PCP4L1	427	SEPTIN5	499	ZNF791
68	CBX2	140	EBNA1BP2	212	HEPACAM2	284	MAN2B1	356	PCSK1	428	SEZ6L	500	ZSCAN9
69	CBX5	141	EIF1	213	HES7	285	MAP1LC3B	357	PDE6H	429	SGF29		
70	CCDC43	142	EIF1B	214	HLA-A	286	MAP3K2	358	PELI2	430	SH3BGRL2		
71	CCDC90B	143	EIF3F	215	HLA-DRB1	287	MAP3K7	359	PET117	431	SHISA3		
72	CCL3L1	144	EIF4A3	216	HMGN3	288	MAPK13	360	PFKM	432	SHPK		

VII. Interpretation of Results:

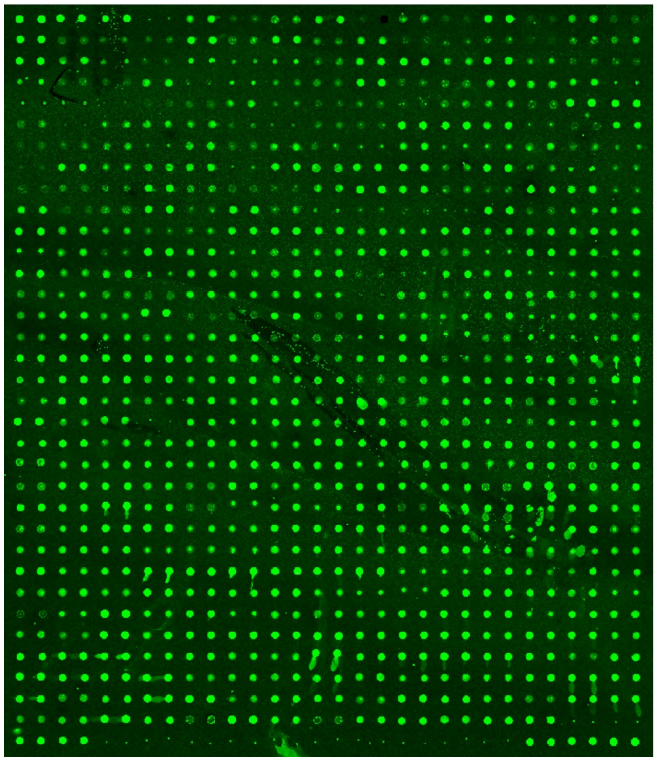
A. Explanation of Controls Spots

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.

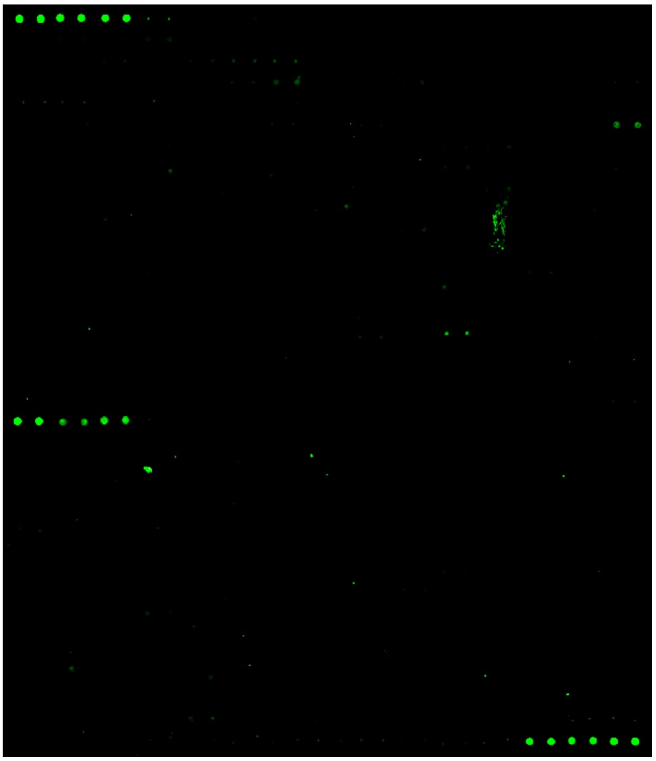
B. Typical Results

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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