RayBio® Label-Based (L-Series) Human L15 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-15-4 (4 Sample Kit)
AAH-BLG-15-8 (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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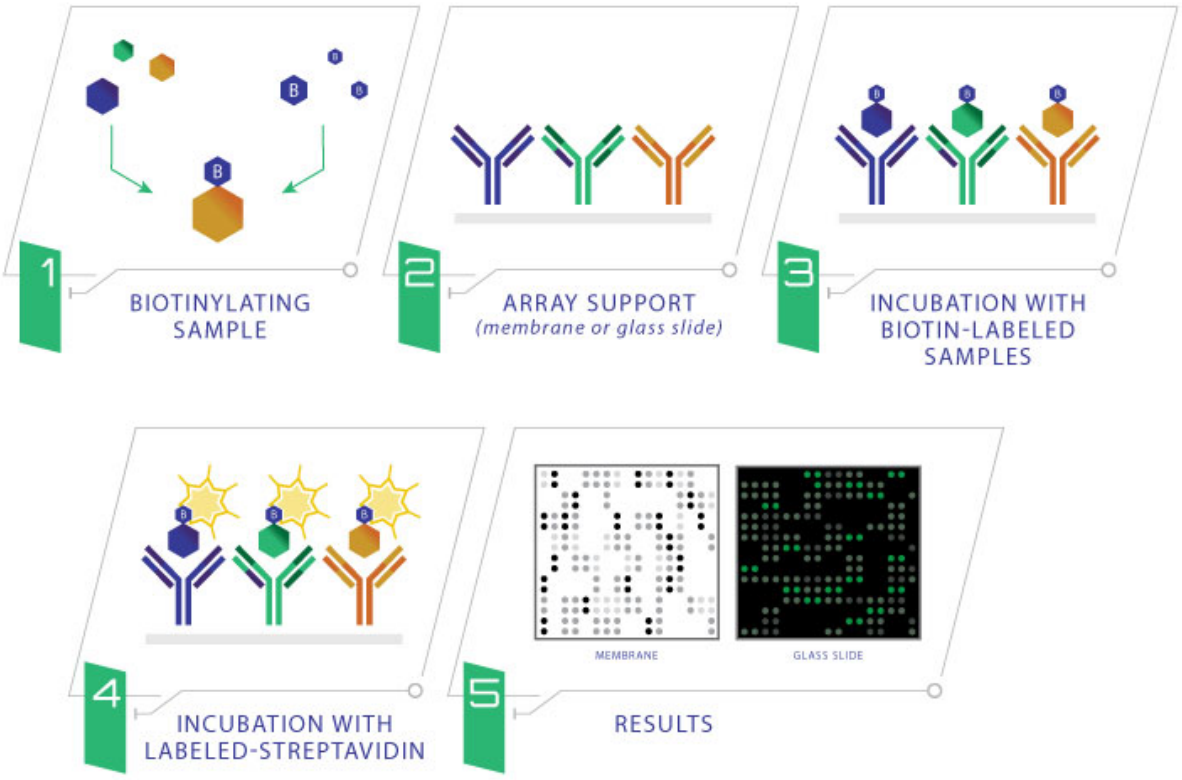
I. Introduction

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.

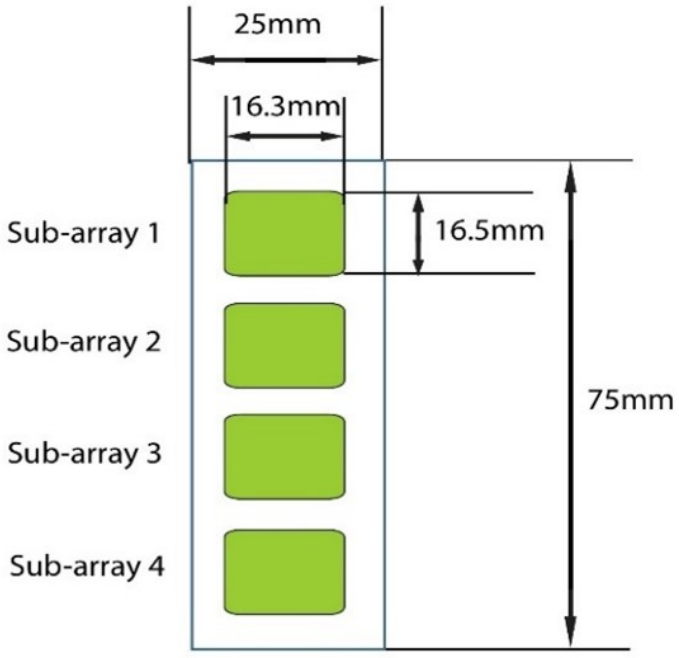
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

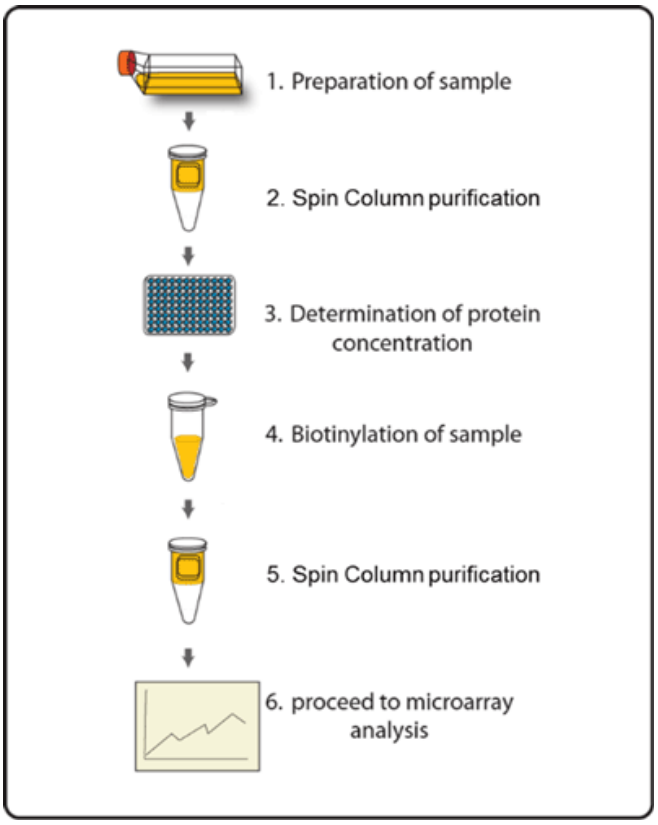
D. Incubations and Washes

- 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.

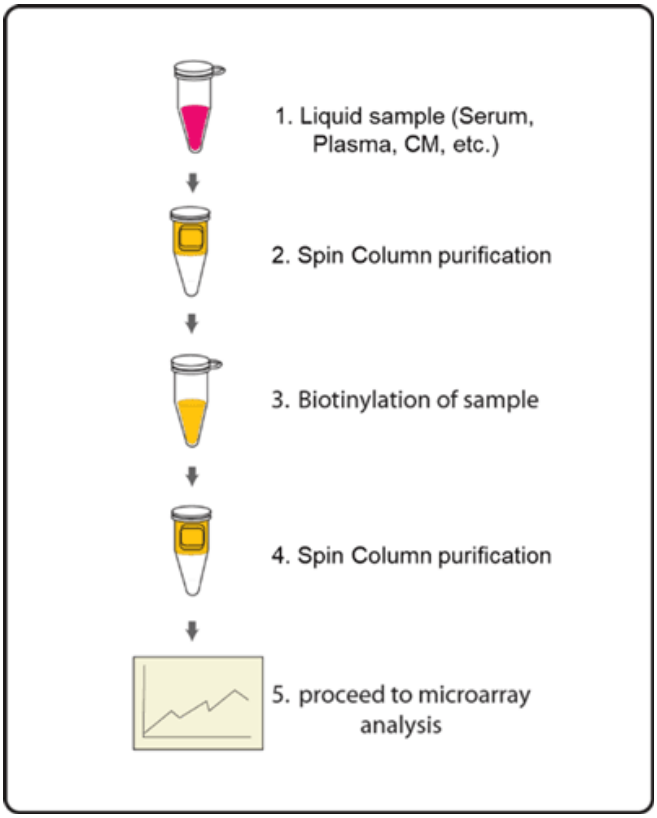
IV. Protocol

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.

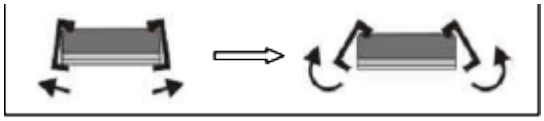
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

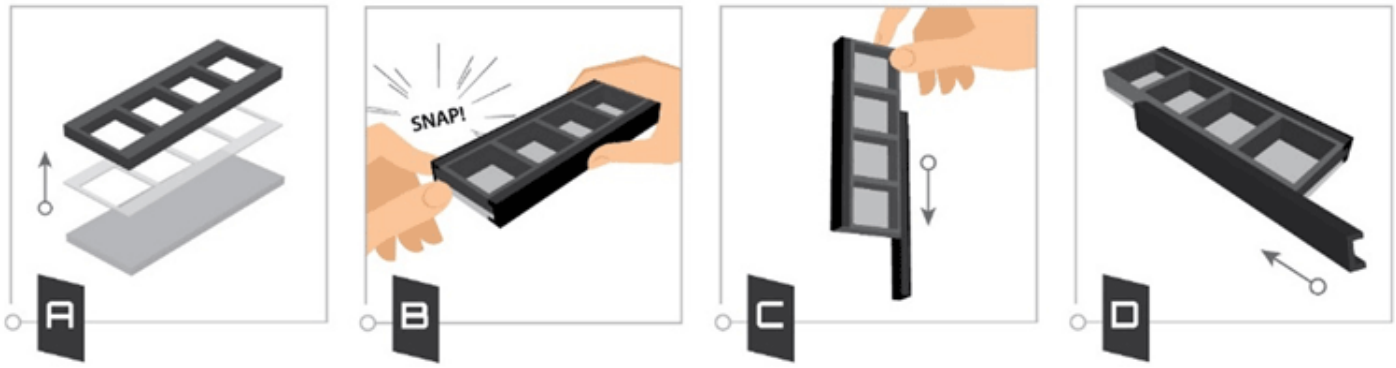
E. Fluorescence Detection

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

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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	ACO2	73	CEACAM20	145	EMP3	217	GRP111	289	MCPIP1	361	PRL3	433	SYT1
2	ACSL4	74	CEP250	146	ENTPD8	218	GRP115	290	MDM4	362	PSGR	434	TACR1
3	ACVR1C	75	CFLAR	147	EPM2A	219	GRP126	291	MESP1	363	PTP4A1	435	TAFA1
4	ACVRL1	76	CHODL	148	ERAL1	220	GSAP	292	METAP1	364	PTPN13	436	TAFA2
5	ADMR	77	CHST5	149	ERC2	221	H2AX	293	METTL1	365	PVALB	437	TAFA3
6	ADORA2B	78	CLEC10A	150	ERMAP	222	HEPACAM	294	MIB1	366	PVRIG	438	TAFA4
7	ADPRH	79	CLEC12A	151	ERRFI1	223	HLA-DR	295	MIB2	367	QPCT	439	TAFA5
8	ADRA1A	80	CLEC1B	152	ETS2	224	HOXD10	296	MLK4	368	RACK1	440	TAGLN
9	Adropin	81	CLEC4A	153	EVI2A	225	HS2ST1	297	MMEL1	369	RAD51	441	TAOK2
10	AIF1	82	CLEC4D	154	EXOC1	226	HSPB8	298	MPC2	370	RARRES3	442	TARM1
11	AKT1S1	83	CLEC4G	155	EXOC4	227	HUNK	299	MPP3	371	RBBP4	443	Tau
12	AKT3	84	CLEC5A	156	EYA3	228	ICAM4	300	MRGPRF	372	REG1B	444	TBX2
13	Allergin-1	85	CLEC6A	157	FANCD2	229	IFNL4	301	MRGX2	373	RGS8	445	TBX20
14	AMIGO1	86	CLEC9A	158	FBXL14	230	IGDCC4	302	MS4A3	374	RLN2	446	TBX6
15	ANXA8	87	CLSPN	159	FBXO22	231	IKBKE	303	MS4A4A	375	RNF128	447	TCF3
16	APCDD1	88	CLUL1	160	FBXO3	232	ILKAP	304	MYOG	376	RNF168	448	TERF1
17	APLP2	89	CRBN	161	FBXO8	233	INSL6	305	NBN	377	RNF4	449	TEX19
18	ARF6	90	CREB3L1	162	FBXW11	234	IP6K1	306	NCR3LG1	378	RPS6KA4	450	TFCP2L1
19	ARHGEF9	91	CRYAB	163	FBXW2	235	IRF3	307	NEUROG1	379	RPS6KA5	451	TH
20	ASAM	92	CSTL1	164	FBXW4	236	IRF8	308	NFIL3	380	RTN4B	452	THAP11
21	ASGR1	93	CUBN	165	FCAMR	237	ISG15	309	NFKB2	381	S100A16	453	TIM-3
22	ASTL	94	CYP3A4	166	FCGRT	238	ISLR2	310	NFKBIB	382	S1PR3	454	TLK2
23	ATG14	95	CYP4Z1	167	FCRL1	239	ITFG1	311	NFKBIE	383	S1PR4	455	TLR8
24	ATG7	96	DCSTAMP	168	FCRL2	240	JADE1	312	NINJ1	384	S1PR5	456	TM4SF18
25	ATP1A3	97	DDOST	169	FCRL3	241	JAK3	313	NLRP1	385	SARM1	457	TMED1
26	ATP7B	98	Dermatopontii	170	FCRL4	242	KCNE4	314	NLRP3	386	SCARA5	458	TMEM119
27	AVPR1A	99	DGCR2	171	FCRL5	243	KEAP1	315	NLRP6	387	SCNN1G	459	TMEM219
28	B3GAT1	100	DGKE	172	FCRL6	244	KHK	316	NPNT	388	SFTPC	460	TMEM87A
29	B7-H7	101	DHFR	173	FCRLB	245	KIF5A	317	NPR1	389	SH3KBP1	461	TNFAIP3
30	BANK1	102	DIDO1	174	FGFBP2	246	KIR3DL3	318	NPY1R	390	SHISA4	462	TOLLIP
31	BATF	103	DISP1	175	FKBP14	247	KIRREL2	319	NR0B2	391	SIGLEC15	463	TPPP2
32	BATF3	104	DNAAF4	176	FKBP1B	248	KLB	320	NR1H3	392	SIGLEC16	464	TPSG1
33	BCL9	105	DNAI1	177	FLT3	249	KLF10	321	NR3C2	393	SIRPB2	465	TRABD2A
34	BCL9L	106	DNAJA4	178	FNDC5	250	KLF17	322	NR4A1	394	SIRPD	466	TRABD2B
35	BIM L	107	DNAL4	179	FOXA2	251	KLRC2	323	NRARP	395	SIT1	467	TREML1
36	BMAL1	108	DNALI1	180	FOXE3	252	KLRC3	324	NTAL	396	SLAMF7	468	TREML2
37	BMI1	109	DNASE1L3	181	FOXF1	253	L1TD1	325	NXPH3	397	SLC11A1	469	TRIM
38	BNIP3	110	DNM2	182	FOXO6	254	LAIR2	326	OTOL1	398	SLC12A5	470	TRIM38
39	BNIP3L	111	DNM3	183	FSCN3	255	LAMP3	327	OTX2	399	SLC14A1	471	TRIM63
40	BORA	112	DNMBP	184	FSHR	256	LAT	328	P2RX1	400	SLC17A6	472	TSHR
41	Brachyury	113	DNMT3L	185	FXYD4	257	LAX1	329	PAG1	401	SLC19A1	473	TSPAN12
42	BSCL2	114	DOC2B	186	FXYD6	258	LCOR	330	PAK2	402	SLC40A1	474	TSPAN33
43	BTN2A2	115	DOK1	187	FZD10	259	LCP2	331	PARL	403	SLC5A5	475	TSPAN8
44	C1QTNF9	116	DOK5	188	FZD2	260	LCTL	332	PAX7	404	SLC5A8	476	TUBA8
45	C2CD3	117	DOK7	189	GABRA4	261	LGR4	333	PCDHGC3	405	SLC9B2	477	TXN2
46	C5AR2	118	DPCR1	190	GABRG1	262	LILRA1	334	PDE7B	406	SLIT3	478	TYROBP
47	CA5A	119	DPEP1	191	GABRG2	263	LILRA6	335	PDE8A	407	SLITRK6	479	UBASH3B
48	CALML3	120	DPP10	192	GAD2	264	LILRB5	336	PDE8B	408	SMC1A	480	UBE2A
49	CCDC158	121	DPP9	193	GAS8	265	LIN28A	337	PDGFRL	409	SMURF1	481	UBE2L6
50	CD161	122	DPPA4	194	GCC1	266	LINGO1	338	PDPN	410	SNTG1	482	UNC13B
51	CD177	123	DPPA5	195	GDAP1	267	LINGO2	339	PDZD11	411	SOCS3	483	VAMP7
52	CD207	124	DRAXIN	196	GDPD2	268	LMTK2	340	PEAR1	412	SOCS7	484	VANGL1
53	CD22	125	DRD5	197	GDPD5	269	LPHN2	341	PGPEP1	413	SOSTDC1	485	Vasohibin
54	CD300B	126	DUSP15	198	GHRHR	270	LRFN1	342	PHPT1	414	SOX1	486	VIP
55	CD300C	127	DUX4	199	GLP1R	271	LRFN3	343	PIK3R3	415	SOX11	487	VSIG2
56	CD300E	128	DVL2	200	GNLY	272	LRFN4	344	PILRB	416	SOX7	488	VSIG8
57	CD300F	129	DYNLT1	201	GNS	273	LRP2	345	PIWIL1	417	SPRED1	489	VSTM2A
58	CD300G	130	DYRK2	202	Goosecoid	274	LRRC4B	346	PLA2G7	418	SR140	490	VSTM4
59	CD302	131	ECE1	203	GPBAR1	275	LRRC52	347	PLIN2	419	SRCRB4D	491	VSTM5
60	CD314	132	ECE2	204	GPR107	276	LRRN1	348	PLTP	420	SSTR3	492	VWC2
61	CD63	133	EEA1	205	GPR15	277	LRRTM3	349	PMFBP1	421	ST6GALNAC5	493	WNK1
62	CD69	134	EED	206	GPR158	278	LRRTM4	350	PNLIPRP1	422	STAMP2	494	WNT10B
63	CD82	135	EFS	207	GPR15L	279	LRTM1	351	PNPLA3	423	STAT1	495	WNT11
64	CD83	136	EIF2B1	208	GPR52	280	LY6K	352	PON3	424	STAT2	496	WWOX
65	CD84	137	EIF2B4	209	GPR61	281	LYPD1	353	Porimin	425	STAT6	497	ZBTB38
66	CD8A	138	EIF4G3	210	GPR65	282	LYPD8	354	PPP2CA	426	STK3	498	ZIC1
67	CDH12	139	ELA2	211	GPR75	283	MALT1	355	PPP3R1	427	STK4	499	ZNF10
68	CDK4	140	ELAVL3	212	GPR88	284	MAPK12	356	PPP4C	428	STRA6	500	ZNF143
69	CDK5R1	141	ELAVL4	213	GPRC5C	285	MAPK3	357	PRDM14	429	STX12		
70	CDKN2A	142	ELFN1	214	GRIN2B	286	MASTL	358	PRKACA	430	STYK1		
71	CDNF	143	EMD	215	GRK2	287	MCEMP1	359	PRKAR2A	431	SUZ12		
72	CEACAM19	144	EMILIN2	216	GRP109A	288	MCM7	360	PRKCD	432	SYNPO		

VII. Interpretation of Results:

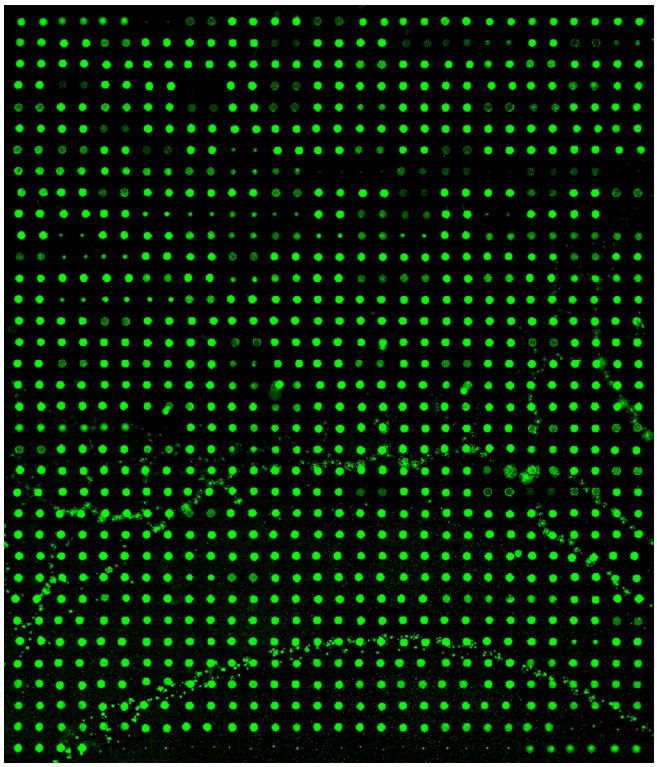
A. Explanation of Controls Spots

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.

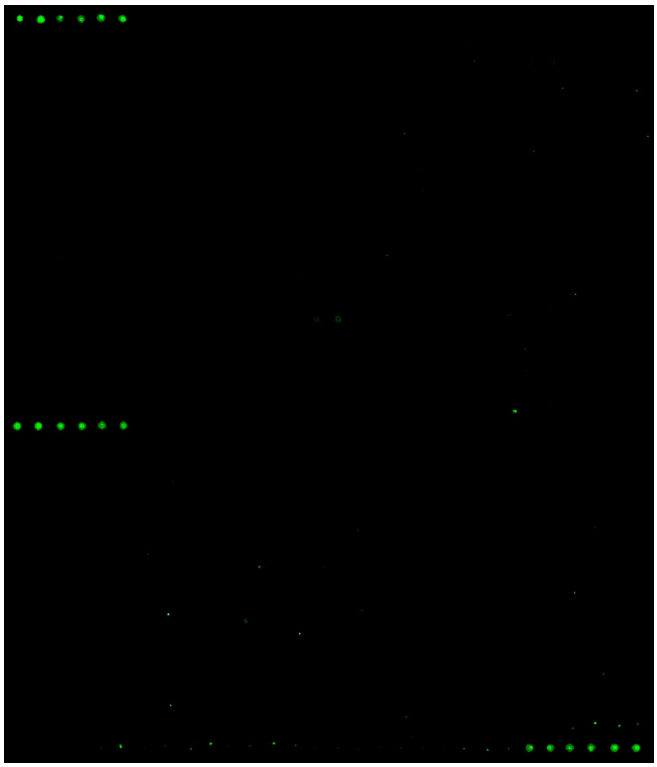
B. Typical Results

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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