RayBio® Label-Based (L-Series) Human L13 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-13-4 (4 Sample Kit)
AAH-BLG-13-8 (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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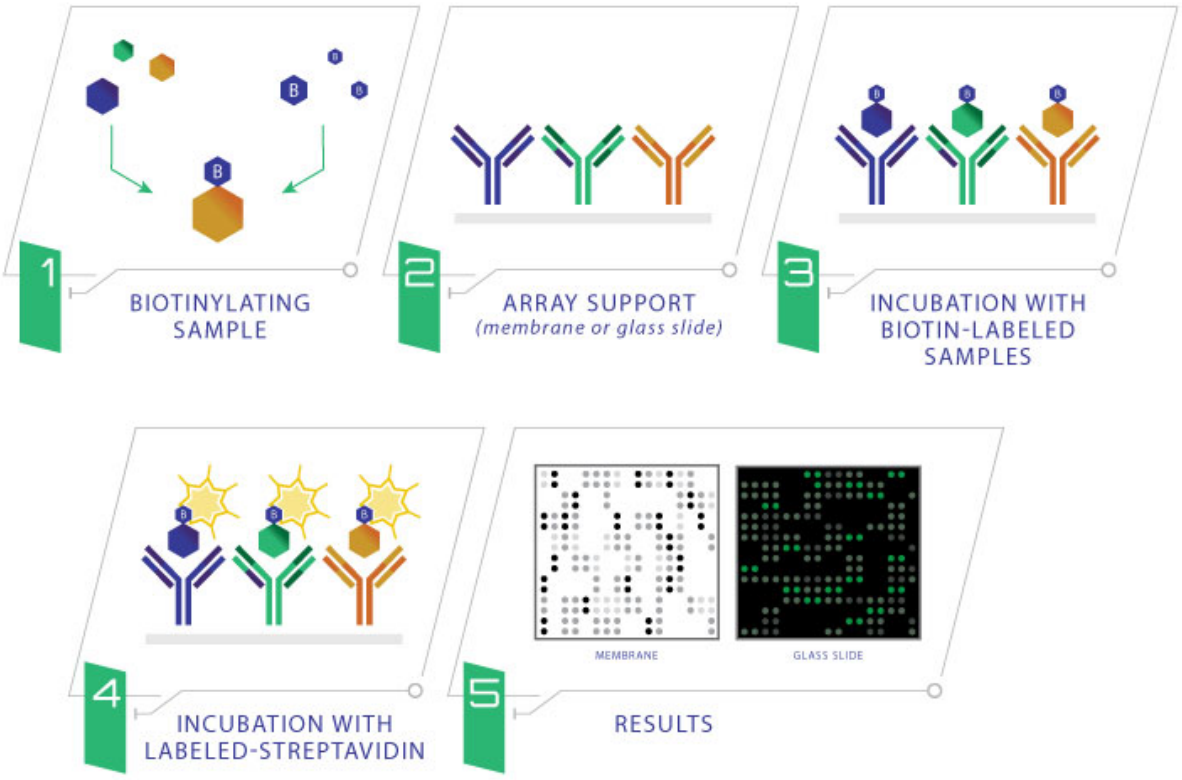
I. Introduction

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.

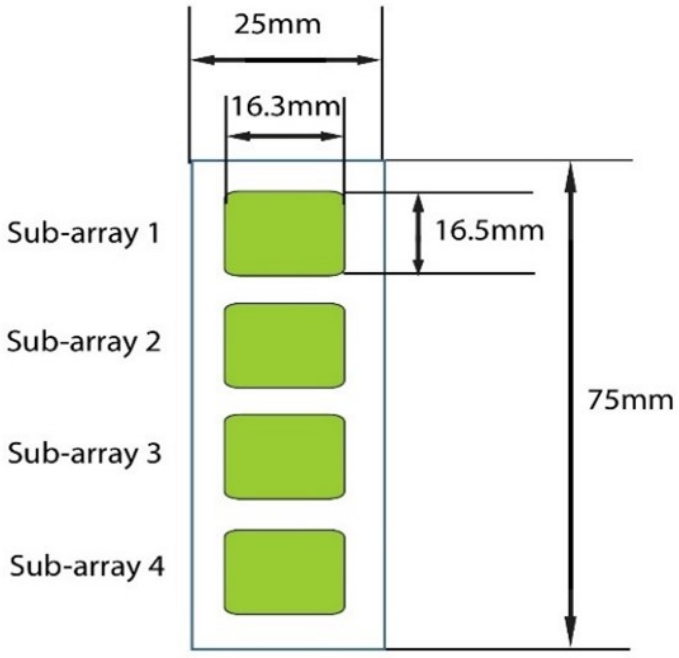
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

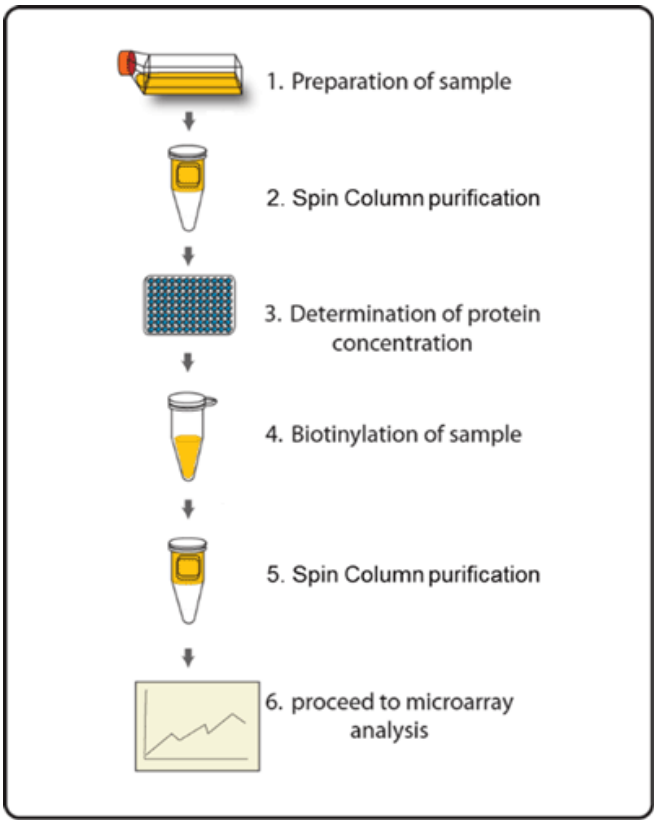
D. Incubations and Washes

- 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.

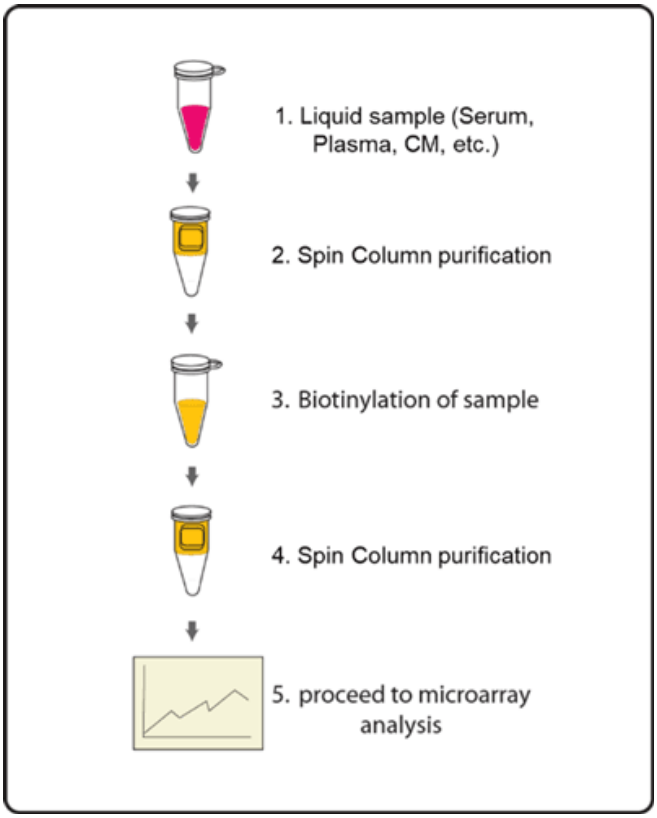
IV. Protocol

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.

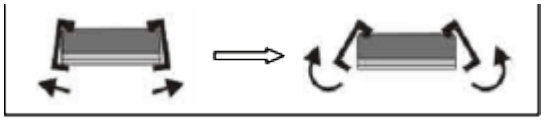
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

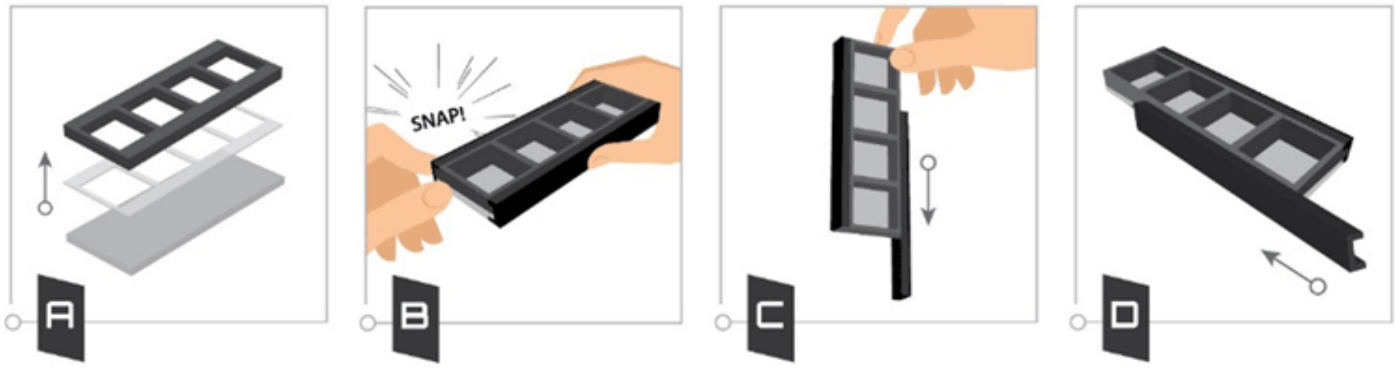
E. Fluorescence Detection

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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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	ACVR2B	73	HLTF	145	MCOLN3	217	NIPBL	289	RAB37	361	SNRPD3	433	TUBB
2	ADAM15	74	HLX	146	MCRS1	218	NIPSNAP3B	290	RAB40B	362	SNRPN	434	TUBB2B
3	ADAM8	75	HMGXB4	147	MEA1	219	NKRF	291	RAB9B	363	SNX19	435	TUBD1
4	AMN	76	HOOK1	148	MEIS3	220	NKTR	292	RAI1	364	SNX7	436	TULP1
5	ARID4A	77	HOOK2	149	MEPE	221	NOD2	293	RASGRP2	365	SOX8	437	TULP4
6	BEGAIN	78	HOXC10	150	METAP2	222	NOVA2	294	RASSF7	366	SP110	438	UBA7
7	BFSP1	79	HOXC4	151	METTL3	223	NPAS1	295	RB1CC1	367	SPAST	439	UBC
8	BIRC8	80	HOXC9	152	MGEA5	224	NPHP4	296	RBFOX2	368	SPATA7	440	UBE2G1
9	BNIPL	81	HOXD12	153	MICAL2	225	NRIP1	297	RBMS2	369	SPIN2B	441	UBE2V1
10	BOLL	82	HOXD13	154	MID2	226	NT5E	298	RBMX2	370	SRY	442	UBE2V2
11	BRPF1	83	HPCAL4	155	MIPOL1	227	NTRK3	299	RBP3	371	SSNA1	443	UBL7
12	BRSK2	84	HPS1	156	MKLN1	228	NXF3	300	RCC1L	372	ST14	444	UCP3
13	CA13	85	HPS5	157	MLC1	229	NXT2	301	RECQL4	373	ST6GAL1	445	ULBP1
14	CACNB1	86	HRASLS5	158	MLXIP	230	ORAI1	302	REG4	374	ST8SIA3	446	UNC5C
15	CACNG3	87	HSD17B1	159	MOB3A	231	OSBPL10	303	REPS2	375	STARD3NL	447	UPK2
16	CALN1	88	HTRA4	160	MRAP	232	OSGIN1	304	RFX5	376	STMN3	448	UQCR
17	CCN1	89	IDO1	161	MRM2	233	OVOL1	305	RFXAP	377	STOML3	449	URB1
18	CD320	90	IFRD2	162	MRPS31	234	PAEP	306	RGR	378	STX10	450	USF2
19	CD33	91	IFT52	163	MRPS34	235	PAPPA2	307	RGS7	379	SUN5	451	USP12
20	CDK16	92	IFT88	164	MS4A6A	236	PARP1	308	RGS9	380	SYNJ2BP	452	USP16
21	CDK18	93	IGKV3-15	165	MS4A7	237	PARP9	309	RHOB	381	SYT12	453	USP20
22	CPA2	94	IL18	166	MSR1	238	PCDHA2	310	RHOXF2	382	SYT3	454	USP22
23	CSF2RB	95	IL9R	167	MTERF1	239	PCDHA3	311	RHPN1	383	SYT9	455	USP28
24	CTSF	96	IRX5	168	MTG2	240	PCDHB12	312	RIN3	384	SYTL4	456	USP3
25	DDC	97	ITGB3BP	169	MTO1	241	PCDHB15	313	RIOK3	385	TADA3	457	USP33
26	DDX25	98	JPH2	170	MTRF1	242	PCSK2	314	RNASE11	386	TAP1	458	USP37
27	DEDD2	99	KAT14	171	MUC2	243	PCSK7	315	RNF6	387	TBC1D5	459	USP42
28	DEFB103A	100	KAT5	172	MYC	244	PDCD1LG2	316	RPGRIP1	388	TCIRG1	460	USP44
29	DESI2	101	KBTBD2	173	MYEOV	245	PDCL	317	RSAD2	389	TCP10L	461	USP49
30	DHRS1	102	KCNA2	174	MYF6	246	PDK2	318	RUSC1	390	TDP1	462	UTP3
31	DIRAS3	103	KCNA4	175	MYH14	247	PDK4	319	RYR2	391	TDRD3	463	VAX2
32	DLX1	104	KCNC4	176	MYL5	248	PELI1	320	SALL2	392	TEKT1	464	VEZT
33	DMC1	105	KCND1	177	MYL7	249	PFN4	321	SAMD4A	393	TEKT2	465	VGLL1
34	DYNC1H1	106	KCNF1	178	MYLIP	250	PGLYRP3	322	SCEL	394	TEX12	466	VIPAS39
35	EFNA3	107	KCNJ1	179	MYLPF	251	PHTF1	323	SCFD2	395	TEX13A	467	VPS41
36	EFNB3	108	KCNJ10	180	MYO1B	252	PICK1	324	SDC4	396	THAP2	468	VRK2
37	EIF3B	109	KCNJ11	181	MYO1D	253	PJA1	325	SDHC	397	THBS3	469	VRK3
38	FBXL4	110	KCNJ13	182	MYO1E	254	PKD2L1	326	SEC22B	398	TIAF1	470	WBP1
39	FOLR2	111	KCNJ6	183	MYOC	255	PKNOX1	327	SECISBP2	399	TIGIT	471	WDR37
40	GABRA1	112	KCNK5	184	N6AMT1	256	PLCD4	328	SEMA6A	400	TIMM23	472	WDR46
41	GFM2	113	KCNS2	185	NAB1	257	PLXND1	329	SEMA6C	401	TINF2	473	WFDC1
42	GIMAP2	114	KCTD5	186	NAE1	258	PNMA1	330	SENP3	402	TLE2	474	WIPF2
43	GIMAP5	115	KDM5A	187	NANOS3	259	PNRC1	331	SENP5	403	TM7SF3	475	WIPI2
44	GJA3	116	KIAA0391	188	NAPB	260	POLR2F	332	SEPTIN2	404	TMC1	476	WSB1
45	GK3P	117	KIF12	189	NCAN	261	POLR2H	333	SEPTIN6	405	TMCC1	477	WSB2
46	GLI4	118	KIF17	190	NCF4	262	POLR2I	334	SEPTIN9	406	TMEM50B	478	XPO1
47	GMEB2	119	KIF1B	191	NCKAP1L	263	POLR2L	335	SERPINB10	407	TMOD4	479	YPEL1
48	GNAI1	120	KIF3C	192	ND1	264	POPDC3	336	SGCG	408	TMPRSS6	480	ZBTB18
49	GNAI3	121	KIR3DL1	193	ND2	265	POTED	337	SGK1	409	TNFAIP1	481	ZBTB40
50	GNAO1	122	KLHL14	194	NDUFA4L2	266	POU2AF1	338	SH3BP5	410	TNNT2	482	ZBTB43
51	GNB2	123	KLHL18	195	NDUFA6	267	POU4F2	339	SHFM1	411	TPPP	483	ZDHHC5
52	GNB3	124	KLHL20	196	NDUFA8	268	PPAN	340	SIGLEC7	412	TRAF7	484	ZHX1
53	GNB4	125	KLHL21	197	NDUFA9	269	PPDPF	341	SIRPA	413	TRAM2	485	ZIC4
54	GNG4	126	KLHL29	198	NDUFB1	270	PPFIA3	342	SIX2	414	TRIM11	486	ZKSCAN8
55	GPR132	127	KLHL3	199	NDUFB10	271	PPM1M	343	SLAMF6	415	TRIM14	487	ZMYND10
56	GPR137B	128	KLHL4	200	NDUFB11	272	PPP2CB	344	SLC10A3	416	TRIM15	488	ZNF136
57	GPSM1	129	KLK13	201	NDUFB2	273	PPP2R2B	345	SLC13A4	417	TRIM17	489	ZNF256
58	GPSM2	130	KLRD1	202	NDUFB3	274	PPP2R2C	346	SLC17A1	418	TRIM25	490	ZNF277
59	GPX5	131	KLRF1	203	NDUFB6	275	PPP3R2	347	SLC18A1	419	TRIM27	491	ZNF3
60	GRK6	132	LAD1	204	NDUFS5	276	PRKACG	348	SLC25A18	420	TRIM39	492	ZNF35
61	GSDMD	133	LAMA4	205	NDUFS7	277	PRKX	349	SLC25A20	421	TRIM41	493	ZNF395
62	GSX1	134	LNX1	206	NECAP1	278	PRLR	350	SLC25A4	422	TRIM46	494	ZNF44
63	GTF3C2	135	LRR1	207	NEDD4L	279	PRMT8	351	SLC25A6	423	TRIM9	495	ZNF443
64	GZMH	136	LRRC41	208	NEK2	280	PRPH2	352	SLC27A3	424	TRMT2A	496	ZNF593
65	HAS3	137	LSM10	209	NELFE	281	PSMA8	353	SLC35A1	425	TROAP	497	ZNF597
66	HBS1L	138	LY6E	210	NFE2	282	PSMD12	354	SLC7A4	426	TRPC4AP	498	ZNF622
67	HCN3	139	LY9	211	NFE2L1	283	PSTPIP2	355	SLC7A8	427	TRPV4	499	ZNF699
68	HINFP	140	LZTR1	212	NFE2L2	284	PTPN18	356	SLC9A8	428	TSEN2	500	ZNHIT2
69	HIP1	141	MAOB	213	NFIA	285	PTPN4	357	SLCO2A1	429	TSKU		
70	HIST3H2A	142	MAP3K7IP2	214	NFKBIA	286	PUS3	358	SLITRK3	430	TSPYL4		
71	HIVEP1	143	MAS1L	215	NFYC	287	RAB1B	359	SMARCD2	431	TTC7A		
72	HIVEP2	144	MCM3AP	216	NGDN	288	RAB24	360	SMARCD3	432	TTC8		

VII. Interpretation of Results:

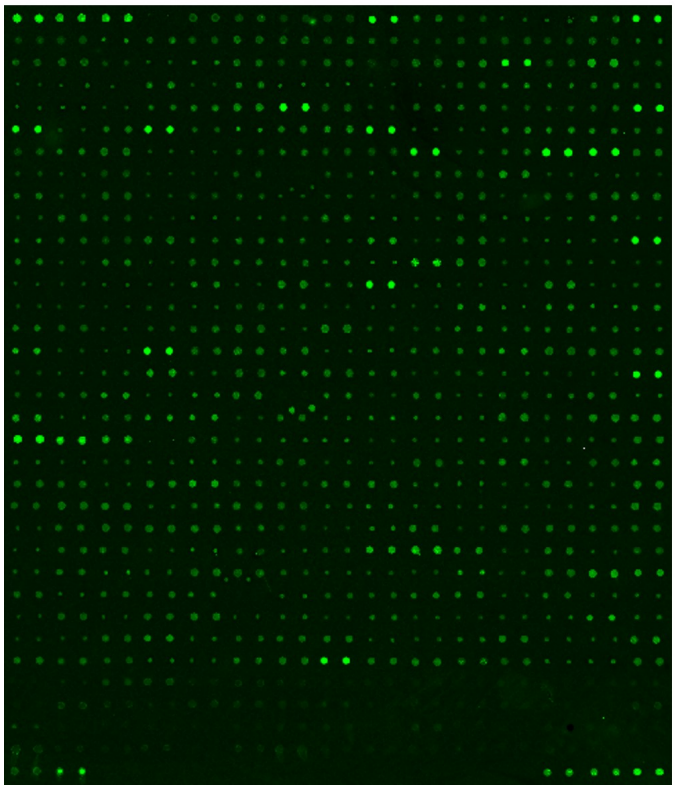
A. Explanation of Controls Spots

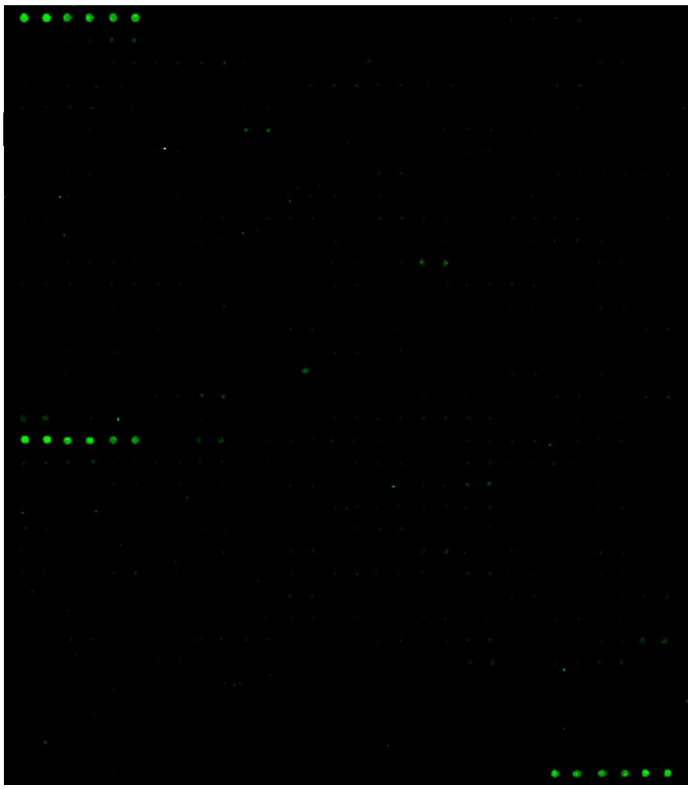
There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized biotinylated IgG. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal 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 Plasma **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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