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

Patent Pending Technology
User Manual (Apr 14, 2023)

For the simultaneous detection of the relative expression of 500 Human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

AAH-BLG-NEU-4 (4 Sample Kit)
AAH-BLG-NEU-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

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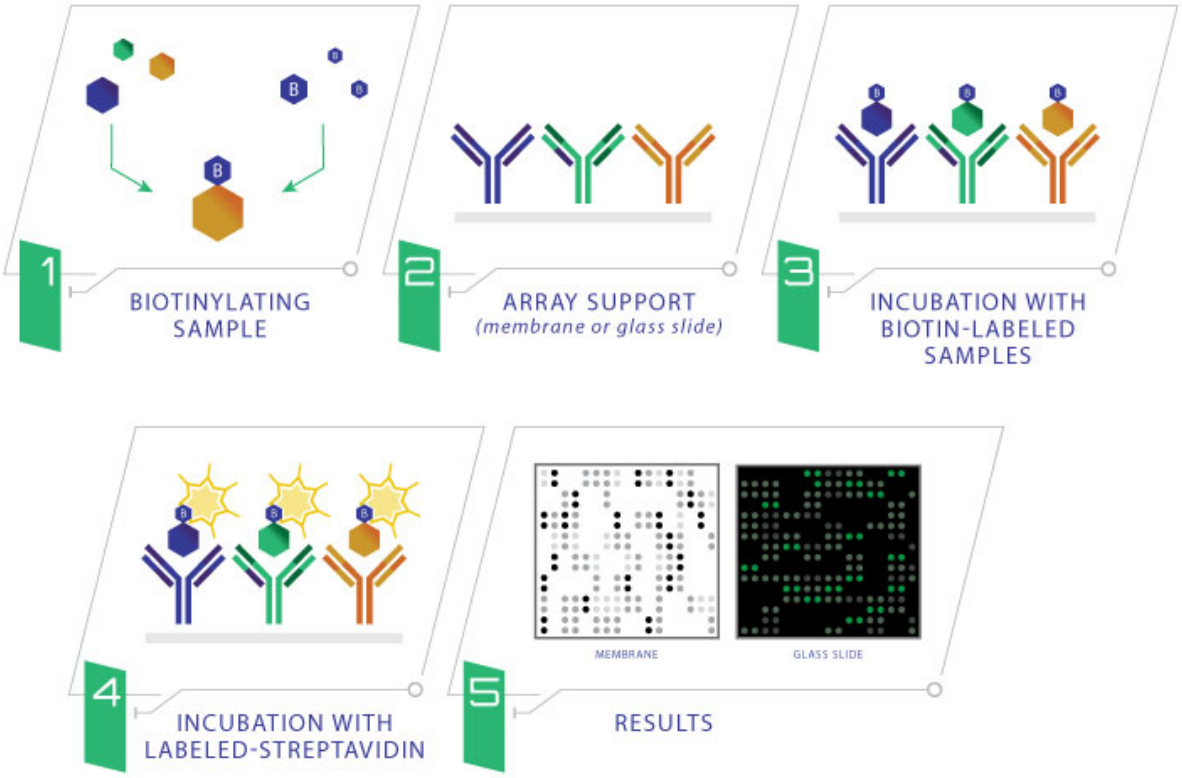
I. Introduction

Combining direct antigen-labeling technology with our vast library of array-validated antibodies, RayBiotech has created the largest commercially available antibody array to date. With the L-Series high density array platform, researchers can now detect thousands of proteins simultaneously, obtaining a broad, panoramic view of protein expression. Our newly expanded panel includes a wide variety of metabolic enzymes, structural proteins, epigenetic markers, neuroregulatory factors, in addition to our popular list of cytokines, growth factors, receptors, adipokines, proteases, and signaling proteins. Available on both glass slide and membrane formats, this array is ideally suited for biomarker discovery studies and exploratory screens.

The first step in using the RayBio® L-Series Antibody Array is to biotinylate the primary amine groups of the proteins in your sample (sera or plasma, cell culture supernatants, cell lysates or tissue lysates). The glass slide arrays are then blocked, just like a western blot, and the biotin-labeled sample is added onto the glass slide, which is pre-printed with capture antibodies. The slide is incubated to allow binding of target proteins. Streptavidin-conjugated fluorescent dye (Cy3 equivalent) is then applied to the array. Finally, the glass slide is dried, and laser fluorescence scanning is used to visualize the signals.



II. Materials Provided

A. Storage Recommendations

Upon receipt, the kit should be stored at -20°C until needed. It is recommended to use the kit within 6 months of the date of shipment. After initial use, remaining reagents should be stored at 4°C and may be stored for up to 3 months. Labeling Reagent (Item B) should be prepared fresh each time before use. Unused glass slides should be kept at -20°C and repeated freeze-thaw cycles should be avoided (slides may be stored for 6 months).

ITEM	DESCRIPTION	4 SAMPLE KIT	8 SAMPLE KIT
A	Spin Columns (0.5ml)	8 columns	16 columns
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50 µl)	1 vial (50 µl)
E	RayBio® L-Series Glass Slide*	1 slide	2 slides
F	Blocking Buffer	1 bottle (8 ml)	2 bottles (8 ml)
G	20X Wash Buffer I	1 bottle (30 ml)	1 bottle (30 ml)
H	20X Wash Buffer II	1 bottle (30 ml)	1 bottle (30 ml)
I	Cy3 equivalent-Conjugated Streptavidin	1 vial	2 vials
J	Adhesive Plastic Strips		
K	Labeling Buffer	1 bottle (30 ml)	1 bottle (30 ml)
n/a	2X Cell Lysis Buffer**	1 bottle (10 ml)	1 bottle (10 ml)
M	30 ml Centrifuge Tube	1 tube	1 tube

*Each slide contains 4 identical subarrays
**Only needed if testing cell or tissue lysates

B. Additional Materials Required

- 1 ml tube, small plastic or glass containers
- Orbital shaker or oscillating rocker
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection
- Aluminum foil

III. Overview and General Considerations

A. Preparation and Storage of Samples

1. Preparation of Cell Culture Supernatants
    1. Seed cells at a density of $1x10^6$ cells in 100 mm tissue culture dishes.*
    2. Culture cells in complete culture medium for ~24-48 hours.**
    3. Replenish with serum-free or low-serum medium such as 0.2% FCS/FBS serum, and then incubate cells again for ~48 hours.**,+ The membrane-based array is recommended if high serum medium such as 10% FCS/FBS is used, as high background can occur on glass slide arrays with high serum containing media samples.
    4. To collect supernatants, centrifuge at 1,000 x g for 10 minutes and store as less than or equal 1 ml aliquots at -80°C until needed.
    5. If you want to use cell mass for inter-sample normalization, measure the total wet weight of cultured cells in the pellet and/or culture dish. You may then normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Or you can normalize between arrays by determining cell lysate concentration using a total protein assay (BCA Protein Assay Kit, Pierce, Prod #: 23227).

    **The density of cells per dish used is dependent on the cell type. More or less cells may be required.*

    ***Optimal culture time may vary and will depend on the cell line, treatment conditions and other factors.*

    +Bovine serum proteins produce detectable signals on the RayBio® L-Series Array in media containing serum concentrations as low as 0.2%. When testing serum-containing media, we strongly recommend testing an uncultured media blank for comparison with sample results.

2. Extracting Protein from Cells
    1. Centrifuging Cells
        a. Adherent Cells:
            i. Remove supernatant from cell culture and wash cells gently twice with cold 1X PBS taking care not to disturb cell layer.
            ii. Add enough cold 1X PBS to cover cell layer and use cell scraper to detach cells.
        b. Cells in Suspension: Pellet the cells by centrifuging using a microcentrifuge at 1500 rpm for 10 minutes.
    2. Make sure to remove any remaining PBS before adding 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O). Solubilize the cells at $2x10^7$ cells/ml in 1X Cell Lysis Buffer.

3. Pipette up and down to resuspend cells and rock the lysates gently at 2-8°C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 13,000 rpm for 10 minutes at 2-8°C.

   Note: If the lysates appear to be cloudy, transfer the lysates to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the lysates are still not clear, store them at -20°C for 20 minutes. Remove from the freezer and immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

4. Transfer lysates to a clean tube. Determining cell lysate concentrations using a total protein assay (BCA Protein Assay Kit, Pierce, Prod# 23227). Aliquot the lysates and store at -80°C.

3. Extracting Protein from Crude Tissue

   1. Transfer approximate 100 mg crude tissue into a tube with 1 ml 1X Cell Lysis Buffer (2X Cell Lysis Buffer should be diluted 2-fold with ddH_2O).
   2. Homogenize the tissue according to homogenizer manufacturer instructions.
   3. Transfer extracts to microcentrifuge tubes and centrifuge for 20 minutes at 13,000 rpm (4°C).

      Note: If the supernatant appears to be cloudy, transfer the supernatants to a clean tube, centrifuge again at 13,000 rpm for 20 minutes at 2-8°C. If the supernatant is still not clear, store the lysate at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

   4. Transfer supernatant to a clean tube and store at -80°C.

4. Determine the total protein concentration

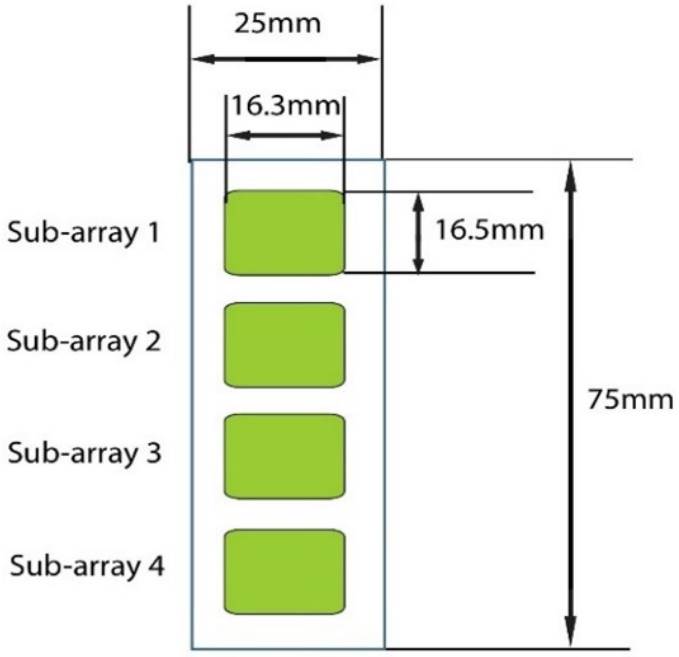
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

B. Handling the Glass Slides

- The microarray slides are delicate. Please do not touch the array surface with pipette tips, forceps or your fingers. Hold the slides by the edges only.
- Handle the slides with powder-free gloves and in a clean environment.
- Do not remove the glass slide from the chamber assembly until step 20, and take great care not to break the glass slide when doing so.
- Remove reagents/sample by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides as seen in image below.

C. Layout of Array Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

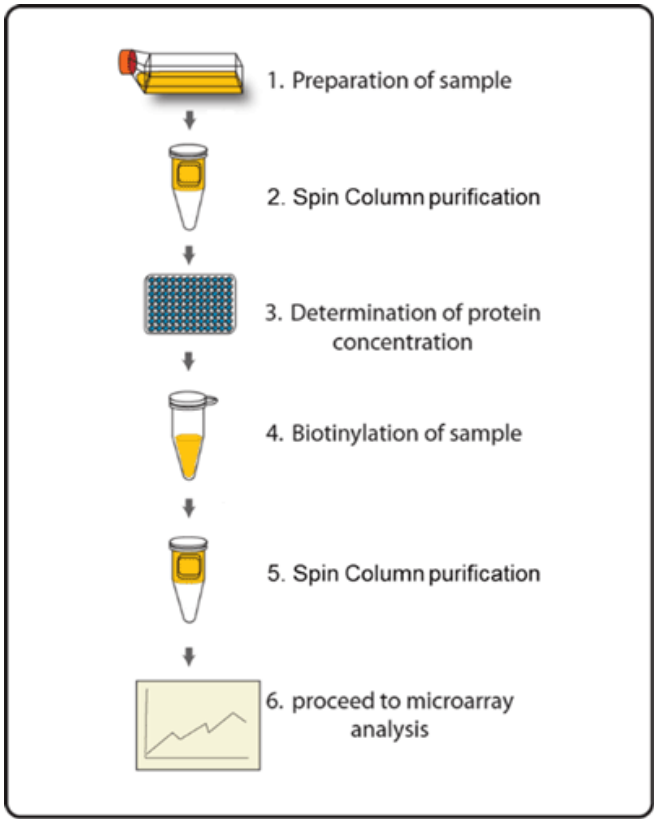
D. Incubations and Washes

- Cover incubation chamber with a Plastic Adhesive Strip (Item J) to prevent evaporation during incubation or wash steps, particularly those steps lasting 2 hours or longer.
- During incubation and wash steps avoid foaming and remove all bubbles from the sub-array surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 4°C
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.
- Unlike most Cy3 fluors, the streptavidin-conjugated fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from direct, strong light and temperatures above RT.

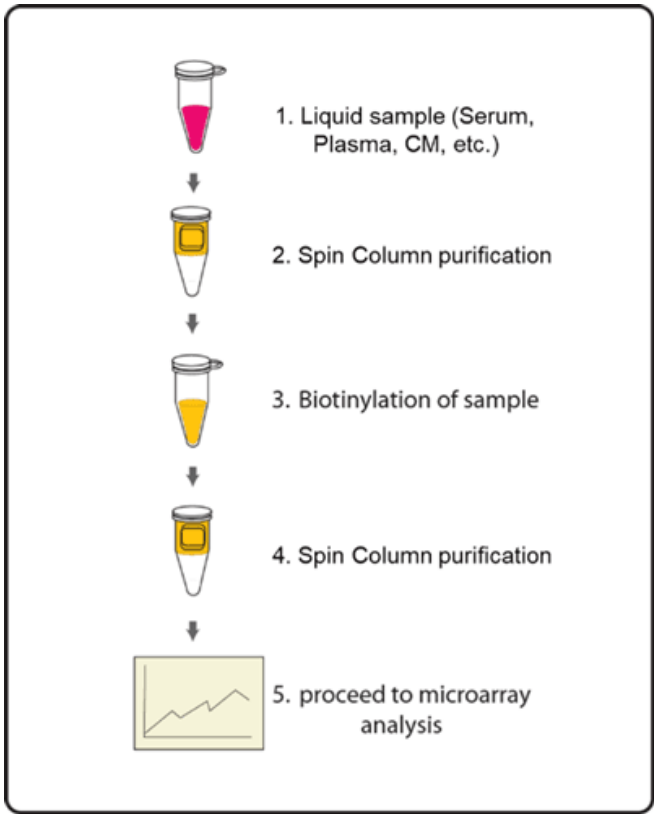
IV. Protocol

Assay Diagram

1. Cell/tissue lysates



2. Serum, plasma, or Cell culture supernatants



A. Sample Purification

Note: This step removes low molecular weight amine derivatives or unwanted buffer from samples to ensure quality biotinylation in Steps 5-7.

1. Twist to remove the bottom plug of the Spin Column and loosen the cap (do not remove).
2. Place the Spin Column into a collection tube and centrifuge at 1,500 x g for 1 minute to remove the storage buffer. Discard the flow-through.
3. Wash the Spin Column three times with 300 µl Labeling Buffer each, centrifuge at 1,500 x g for 1 minute to remove the flow-through. Discard the flow-through and blot the bottom of the column to remove excess liquid. Transfer the Spin Column to a new collection tube.
4. Apply sample on top of the resin within the next few minutes. Centrifuge at 1,500 x g for 2 minutes. Collect the flow-through that contains the sample. The recommended sample dilutions are as follows:

- *Cell culture supernatant: 120 µl neat supernatant*
- *Serum/Plasma: 2 µl serum/plasma in 100 µl Labeling Buffer*
- *Cell/tissue lysate: 20 µg lysate in 100 µl Labeling Buffer*

Note: Each labelled sample volume is enough for at least 3 arrays following the protocol below.

Note: The maximal sample volume is 130 µl for each Spin Column. Do not load over 130 µl of sample into a Spin Column.

B. Biotin Labeling the Sample

Note: Amines (e.g., Tris, glycine) and azides quench the biotinylation reaction. Avoid contaminating samples with these chemicals prior to biotinylation.

5. Immediately before use, prepare the Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 µl Labeling Buffer into the tube, then pipette up and down or vortex slightly to dissolve the lyophilized reagent.
6. Add Labeling Reagent to the sample tube. Incubate the reaction solution at RT with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 minutes.
   a. For labeling cell culture supernatants: Add 8 µl of Labeling Reagent into the sample tube (for 120 µl supernatant).
   b. For labeling serum or plasma: Add 8 µl of Labeling Reagent into the sample tube (for 2 µl serum/plasma *in 100 µl labeling buffer*).
   c. For labeling cell or tissue lysates: Add 4 µl of 1X Labeling Reagent into the sample tube (for 20 µg lysate *in 100 µl labeling buffer*).
   d. For all other body fluid: Add 2 µl of Labeling Reagent Solution per 100 µg sample to be labelled.

   Note: The addition of Labeling Reagent volume is based upon the sample amount used in Step 4. If the amount of sample being labelled differs from the example in Step 6, adjust this volume proportionally.
7. Add 3 µl Stop Solution (Item D) to each sample tube. Using a new spin column, repeat Steps 1-4 of section A. Sample Purification to remove the excess non-reacted biotin reagent from each sample.

   Note: Biotinylated samples can be stored at -20°C or -80°C until you are ready to proceed with the assay.

C. Drying the Glass Slide

8. Remove the package containing the Assembled Glass Slide (Item E) from the freezer. Place unopened package on the bench top for ~15 minutes, and allow the Assembled Glass Slide to equilibrate to RT.

9. Open package, and take the Assembled Glass Slide out of the sleeve. Do not disassemble the Glass Slide from the chamber assembly. Place glass slide assembly in laminar flow hood or similar clean environment for 1-2 hours at RT.

   Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubations

Note: Glass slide should be completely dry before adding Blocking Buffer to wells.

10. Block sub-arrays by adding 400 µl of Blocking Buffer (Item F) into each well of Assembled Glass Slide and incubating at RT for 30 minutes. Ensure there are no bubbles on the array surfaces.
11. Dilute samples with Blocking Buffer. Recommended dilution of the biotin-labeled samples with Blocking Buffer is 10-fold for cell culture supernatants, 20-fold for serum/plasma and 100-fold for cell/tissue lysate. *Dilution for other body fluid needs to be determined by the end user. Generally, most samples can be 10-20x dilution, while tears and saliva samples may need 100x dilution.*

    Note: Optimal sample dilution factor will depend on the abundance of target proteins. If the background or antigen-specific antibody signals are too strong, the sample can be diluted further in subsequent experiments. If the signal is too weak, more concentrated samples can be used.
12. Completely remove the Blocking Buffer from each well. Add 400 µl of diluted sample into appropriate wells. Remove any bubbles on array surfaces. Incubate arrays with gentle rocking or shaking for 2 hours at RT or overnight at 4°C

    Note: Avoid the flow of sample into neighboring wells.
13. Based on number of samples and remaining protocol, calculate the amount of 1X Wash Buffer I and 1X Wash Buffer II needed to complete the experiment. Separately dilute the required amounts of 20X Wash Buffer I Concentrate (Item G) 20-fold and 20X Wash Buffer II Concentrate (Item H) with ddH_2O
14. Decant the samples from each well and wash 3 times with 800 µl of 1X Wash Buffer I at RT with gentle rocking or shaking for 5 minutes per wash.
15. Obtain a clean container (e.g., pipette tip box or slide-staining jar), place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer I to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 10 minutes per wash.
16. Decant the Wash Buffer I from each well, place the Assembled Glass Slide into the container with enough volume of 1X Wash Buffer II to completely cover the entire assembly, and remove any bubbles in wells. Wash 2 times at RT with gentle rocking or shaking for 5 minutes per wash.
17. Prepare 1X Cy3-Conjugated Streptavidin:
    a. Briefly spin down tube containing the Cy3-Conjugated Streptavidin (Item I) immediately before use.

b. Add 1000 µl of Blocking Buffer into the Cy3-Conjugated Streptavidin tube to prepare a concentrated Cy3-Conjugated Streptavidin stock solution. Pipette up and down to mix gently (do not store the stock solution for later use).

c. To prepare 1X Cy3-Conjugated Streptavidin, add 200 µl of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800 µl of Blocking Buffer. Mix gently.

18. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400 µl of 1X Cy3-Conjugated Streptavidin to each sub-array. Cover the incubation chamber with the plastic adhesive strips.

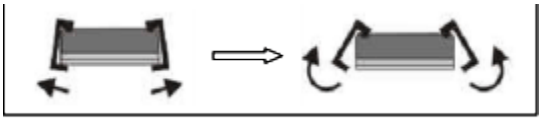
    Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

19. Incubate with 1X Cy3-Conjugated Streptavidin at RT for 1 hour with gentle rocking or shaking.

    Note: Incubation may be done overnight at 4°C

20. Decant the solution and disassemble the glass slide from the incubation frame and chamber. Disassemble the device by pushing clips outward from the side, as shown below. Carefully remove the glass slide from the gasket.

    Note: Be careful not to touch the printed surface of the glass slide, which is on the same side as the barcode.



21. Gently place the glass slide into 30 ml Centrifuge Tube (Item M). Add enough 1X Wash Buffer I to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 10 min. Remove the wash buffer. Repeat 2 times for a total of 3 washes.

22. Add enough 1X Wash Buffer II to cover the entire glass slide (about 30 ml). Wash with gentle rocking or shaking for 5 minutes. Remove the wash buffer. Repeat one time for a total of two washes for 5 minutes per wash.

23. Finally, wash the glass slide with 30 ml of ddH_2O for 5 minutes. Remove glass slide and decant water from Centrifuge Tube.

24. Remove buffer droplets from the slide completely by one of the following ways:
    - Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuge at 1,000 rpm for 3 minutes without cap.
    - Or dry the glass slide by a compressed N2 stream.
    - Or gently apply suction with a pipette to remove buffer droplets. Do not touch the array surface, only the sides.

    Note: Make sure the finished glass slide is completely dry before scanning or storage.

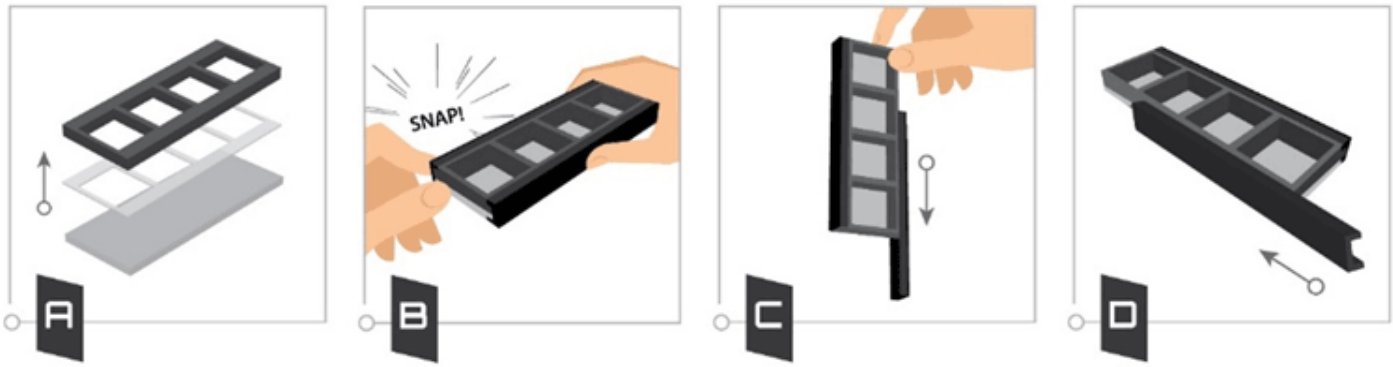
E. Fluorescence Detection

25. You may proceed immediately to scanning or you may store the slide at -20°C in the Centrifuge Tube provided or at RT to scan at a later time.

    Note: Please protect the finished glass slides from temperatures above RT and store them in the dark. Do not expose glass slide to strong light, such as sunlight or a UV lamp.

    Note: If you need to repeat any of the incubation steps after finishing the experiment, you must first re-assemble the glass slide into the incubation chamber by following the steps as described below. To avoid breaking the printed glass slide, you may first want to practice assembling the device with a blank glass slide.

    1. *Apply slide to incubation chamber barcode facing upward (image A).*
    2. *Gently snap one edge of a snap-on side (image B).*
    3. *Gently press other of side against lab bench and push in lengthwise direction (image C).*
    4. *Repeat with the other side (image D)*



V. Antibody Array Map

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1	POS1	POS1	POS2	POS2	POS3	POS3	Neg	Neg	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11
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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	ACTR10	73	CDK5R1	145	FZD2	217	KCNJ6	289	NDUFV1	361	PSMA4	433	STX1A
2	ACTR1A	74	CHRM1	146	FZD3	218	KCNJ9	290	NDUFV2	362	PSMA5	434	TAF4
3	ACTR1B	75	CHRM3	147	FZD4	219	KIF5A	291	NEFH	363	PSMA6	435	TAF4B
4	ADAM10	76	CHRM5	148	FZD5	220	KIF5B	292	NEFL	364	PSMA7	436	TBP
5	ADAM17	77	CHRNA7	149	FZD6	221	KIF5C	293	NEFM	365	PSMA8	437	TFAM
6	ADCY1	78	CHUK	150	FZD7	222	KLC1	294	NFE2L2	366	PSMB1	438	TGM2
7	ADCY2	79	CLTB	151	GABRA1	223	KLC2	295	NFKB1	367	PSMB2	439	TH
8	ADCY3	80	CLTC	152	GABRA4	224	KLC3	296	NGF	368	PSMB3	440	TNFRSF1A
9	ADCY4	81	CLTCL1	153	GABRA5	225	KLC4	297	NOS1	369	PSMB4	441	TNFRSF1B
10	ADCY5	82	COX4I1	154	GABRB2	226	KRAS	298	NOS2	370	PSMB5	442	TOMM40
11	ADCY6	83	COX4I2	155	GABRD	227	LPL	299	NOTCH1	371	PSMB6	443	TOMM40L
12	ADCY7	84	COX5A	156	GABRE	228	LRP1	300	NOX1	372	PSMB7	444	TP53
13	ADCY8	85	COX5B	157	GABRG1	229	LRP5	301	NOX4	373	PSMC2	445	TRAF2
14	ADCY9	86	COX6A1	158	GABRG2	230	LRP6	302	NRAS	374	PSMC3	446	TRPC1
15	ADORA2A	87	COX6A2	159	GABRQ	231	LRRK2	303	NRBF2	375	PSMC4	447	TUBA1A
16	ADRM1	88	COX6B1	160	GABRR2	232	MAOA	304	NRF1	376	PSMC5	448	TUBA1B
17	AKT1	89	COX7A1	161	GAPDH	233	MAOB	305	PIK3C3	377	PSMC6	449	TUBA1C
18	AKT2	90	COX7A2L	162	GLUL	234	MAP2K1	306	PIK3CA	378	PSMD1	450	TUBA3C
19	AKT3	91	COX7B	163	GNAI1	235	MAP2K2	307	PIK3CB	379	PSMD11	451	TUBA4A
20	AMBRA1	92	COX7B2	164	GNAI2	236	MAP2K3	308	PIK3CD	380	PSMD12	452	TUBA8
21	AP2A1	93	COX7C	165	GNAI3	237	MAP2K7	309	PIK3R1	381	PSMD13	453	TUBAL3
22	AP2A2	94	COX8A	166	GNAL	238	MAP3K10	310	PIK3R2	382	PSMD14	454	TUBB
23	AP2B1	95	CREB1	167	GNAO1	239	MAP3K5	311	PIK3R3	383	PSMD2	455	TUBB1
24	AP2M1	96	CREB3L1	168	GNB1	240	MAPK1	312	PIK3R4	384	PSMD3	456	TUBB2B
25	AP2S1	97	CREB3L2	169	GNB2	241	MAPK11	313	PINK1	385	PSMD4	457	TUBB3
26	APAF1	98	CSF1	170	GNB3	242	MAPK12	314	PLA2G4A	386	PSMD6	458	TUBB6
27	APBB1	99	CSNK1A1	171	GNB4	243	MAPK13	315	PLA2G4F	387	PSMD7	459	TXN
28	APH1A	100	CSNK1E	172	GNB5	244	MAPK14	316	PLCB1	388	PSMD8	460	TXN2
29	APOE	101	CSNK2A1	173	GNG11	245	MAPK3	317	PLCB2	389	PSMD9	461	UBA52
30	APP	102	CSNK2A2	174	GNG12	246	MAPK8	318	PLCB3	390	PTGS2	462	UBA7
31	ARAF	103	CTNNB1	175	GNG13	247	MAPK9	319	PLCB4	391	RAB5A	463	UBB
32	ATF4	104	CYC1	176	GNG4	248	MAPT	320	PLCG1	392	RAC1	464	UBC
33	ATF6	105	CYCS	177	GNG5	249	MFN1	321	POLR2A	393	RAF1	465	UBE2G1
34	ATG2A	106	DAXX	178	GNG8	250	MFN2	322	POLR2B	394	RAP1A	466	UBE2G2
35	ATP2A1	107	DCTN1	179	GNGT1	251	MME	323	POLR2C	395	RAP1B	467	UBE2J1
36	ATP2A3	108	DCTN2	180	GNGT2	252	NAE1	324	POLR2D	396	RB1CC1	468	UBE2J2
37	ATP5D	109	DCTN3	181	GPR37	253	NCAM1	325	POLR2E	397	RCOR1	469	UBE2L6
38	AXIN1	110	DCTN4	182	GPR83	254	NCSTN	326	POLR2F	398	RELA	470	UCHL1
39	AXIN2	111	DCTN5	183	GPX1	255	NDUFA1	327	POLR2G	399	REST	471	UCP1
40	BACE1	112	DCTN6	184	GPX2	256	NDUFA10	328	POLR2H	400	RPS27A	472	ULK2
41	BACE2	113	DDC	185	GPX3	257	NDUFA12	329	POLR2I	401	RPS6KA1	473	UQCRB
42	BAD	114	DDIT3	186	GPX5	258	NDUFA13	330	POLR2J	402	RPS6KA2	474	UQCRC1
43	BAX	115	DERL1	187	GPX7	259	NDUFA2	331	POLR2J2	403	RPS6KA3	475	UQCRC2
44	BCL2	116	DKK1	188	GRIA1	260	NDUFA3	332	POLR2K	404	RPS6KA6	476	UQCRFS1
45	BCL2L1	117	DKK2	189	GRIA2	261	NDUFA4	333	POLR2L	405	RTN3	477	UQCRH
46	BDNF	118	DKK4	190	GRIN1	262	NDUFA4L2	334	PPARG	406	RTN4	478	UQCRQ
47	BECN1	119	DLG4	191	GRIN2A	263	NDUFA5	335	PPARGC1A	407	SDHB	479	VDAC1
48	BID	120	DNAH17	192	GRIN2B	264	NDUFA6	336	PPID	408	SDHC	480	VDAC2
49	BRAF	121	DNAH8	193	GRIN2C	265	NDUFA7	337	PPIF	409	SIN3A	481	WIPI1
50	C1QA	122	DNAI1	194	GRIN2D	266	NDUFA8	338	PPP1CA	410	SLC17A6	482	WIPI2
51	C1QC	123	DNAI2	195	GRM1	267	NDUFA9	339	PPP1CC	411	SLC17A7	483	WNT1
52	C5	124	DNAL1	196	GRM5	268	NDUFAB1	340	PPP2CA	412	SLC18A1	484	WNT10A
53	C6	125	DNAL4	197	GSK3B	269	NDUFB1	341	PPP2CB	413	SLC18A2	485	WNT10B
54	C7	126	DNALI1	198	HAP1	270	NDUFB10	342	PPP2R1A	414	SLC1A1	486	WNT11
55	C8B	127	DRD1	199	HDAC1	271	NDUFB11	343	PPP2R1B	415	SLC1A2	487	WNT16
56	CACNA1C	128	DRD2	200	HDAC2	272	NDUFB2	344	PPP3CA	416	SLC1A3	488	WNT2
57	CACNA1S	129	DUSP1	201	HIP1	273	NDUFB3	345	PPP3CB	417	SLC25A31	489	WNT2B
58	CALM1	130	DVL1	202	HRAS	274	NDUFB4	346	PPP3R1	418	SLC25A4	490	WNT3A
59	CALM2	131	DVL2	203	HSPA1A	275	NDUFB5	347	PPP3R2	419	SLC25A5	491	WNT4
60	CALML3	132	DVL3	204	HSPA5	276	NDUFB6	348	PRKACA	420	SLC25A6	492	WNT5A
61	CALML5	133	EGR1	205	HTRA2	277	NDUFB7	349	PRKACB	421	SLC32A1	493	WNT5B
62	CAMK2A	134	EIF2AK2	206	IDE	278	NDUFB8	350	PRKACG	422	SLC38A1	494	WNT6
63	CAMK2B	135	EIF2AK3	207	IFT57	279	NDUFB9	351	PRKCA	423	SLC38A2	495	WNT7A
64	CAMK2D	136	EIF2S1	208	IKBKB	280	NDUFC1	352	PRKCB	424	SLC38A3	496	WNT7B
65	CAMK4	137	ELK1	209	IL1A	281	NDUFS1	353	PRKCG	425	SLC6A1	497	WNT8B
66	CASP1	138	EP300	210	IL1B	282	NDUFS2	354	PRPH	426	SLC6A3	498	WNT9A
67	CASP3	139	ERN1	211	IL6	283	NDUFS3	355	PSEN1	427	SNCA	499	WNT9B
68	CASP7	140	FADD	212	INSR	284	NDUFS4	356	PSEN2	428	SNCAIP	500	XBP1
69	CASP8	141	FAS	213	IRS1	285	NDUFS5	357	PSENEN	429	SOD1		
70	CASP9	142	FOS	214	KCNJ10	286	NDUFS6	358	PSMA1	430	SOD2		
71	CAT	143	FRAT2	215	KCNJ3	287	NDUFS7	359	PSMA2	431	SP1		
72	CCL5	144	FZD1	216	KCNJ5	288	NDUFS8	360	PSMA3	432	STIP1		

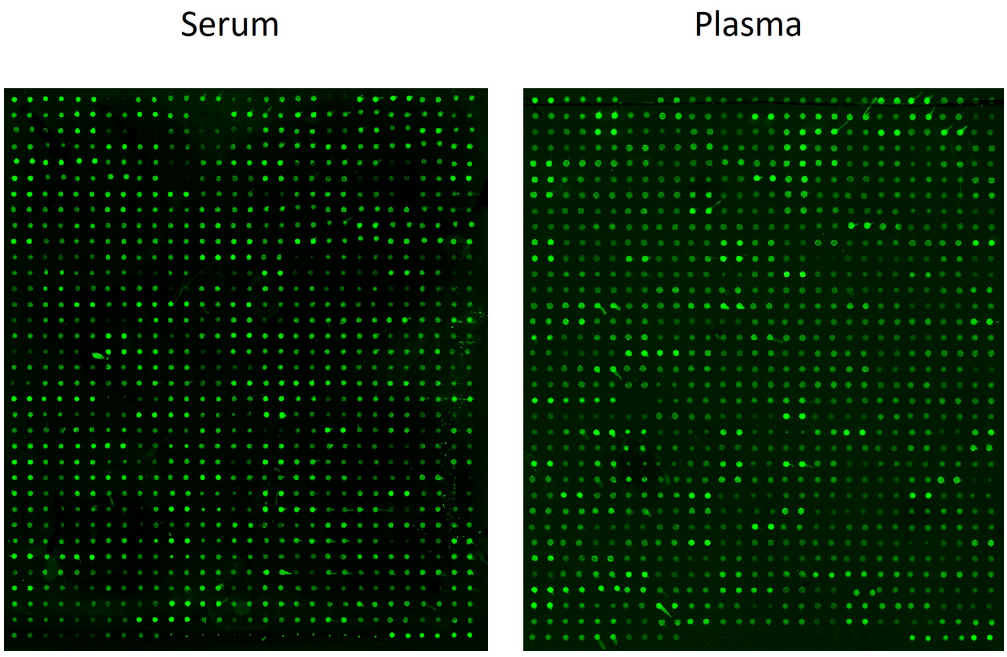
VII. Interpretation of Results

A. Explanation of Controls Spots

There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized biotinylated IgG. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal responses to a known control, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

B. Typical Results

The following figure shows the typical result of this array probed with sample(s). The images were captured using an Axon GenePix laser scanner. The Positive control signals in the upper left and lower right corners of each array can be used to identify the orientation and help normalize the results between arrays.



Note: In the absence of an external standard curve for each protein detected, there is no means of assessing absolute or relative concentrations of different proteins in the same sample using immunoassays. If you wish to obtain quantitative data (i.e., concentrations of the various analytes in your samples), try using our Quantibody® Arrays as a targeted follow-up experiment.

C. Background Subtraction

Once you have obtained fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Most laser fluorescence scanners' software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEAN signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "F532 Mean - B532"), you may need to subtract the background manually or change the default settings on your scanner's data report menu.

D. Normalization of Array Data

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary. For example, in our Analysis Tool Software (described below), the array represented by data entered in the left-most column each worksheet is the default "reference array."

You can calculate the normalized values as follows:

$X(Ny) = X(y) * P1/P(y)$

Where:
P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is freely available for use with data obtained using RayBio® Biotin Label-based Antibody Arrays. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls. Analysis Tool software can be downloaded from the product page on the RayBiotech website.

E. Threshold of Significant Difference

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences in expression levels of each protein between samples or groups.

Any greater than or equal to 1.5-fold increase or less than or equal to 0.65-fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy is around 95%).

VIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Dilute starting sample less or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven Signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completed covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
General	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated
High Background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from dying out during experiment

IX. Selected References

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