RayBio® Label-Based (L-Series) Human Cancer 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-CAN-4 (4 Sample Kit)
AAH-BLG-CAN-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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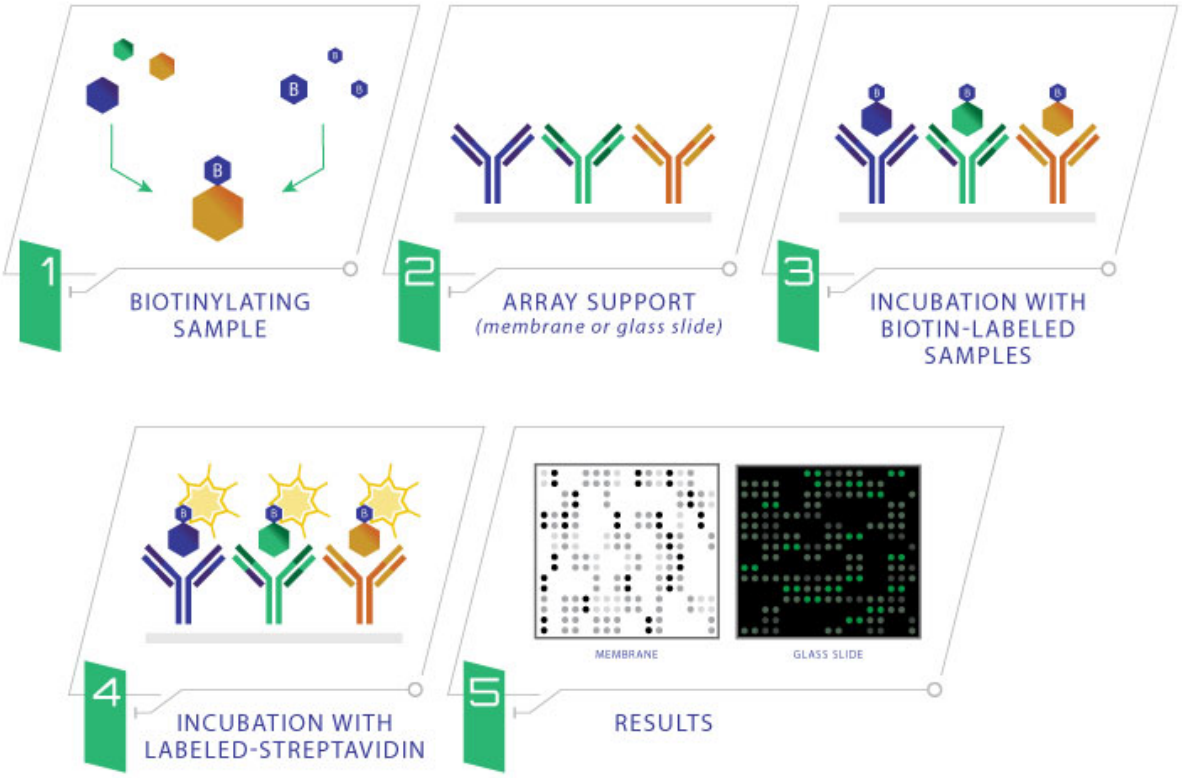
I. Introduction

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

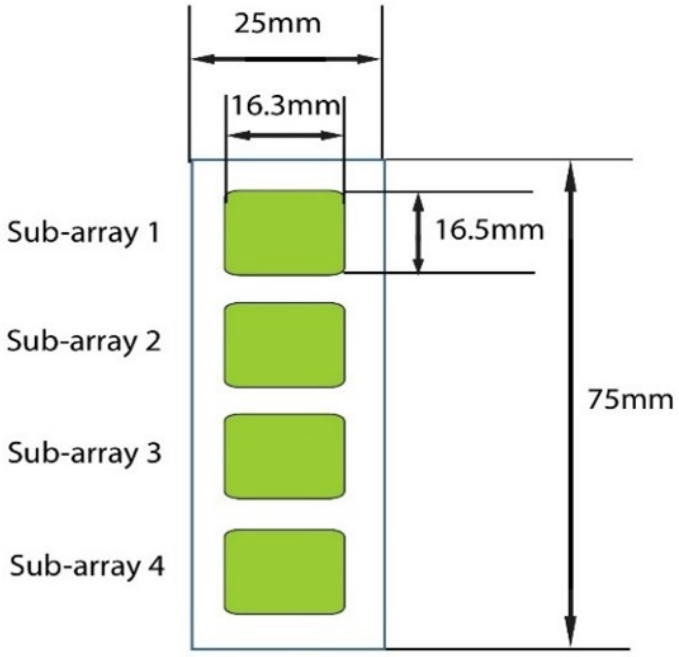
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

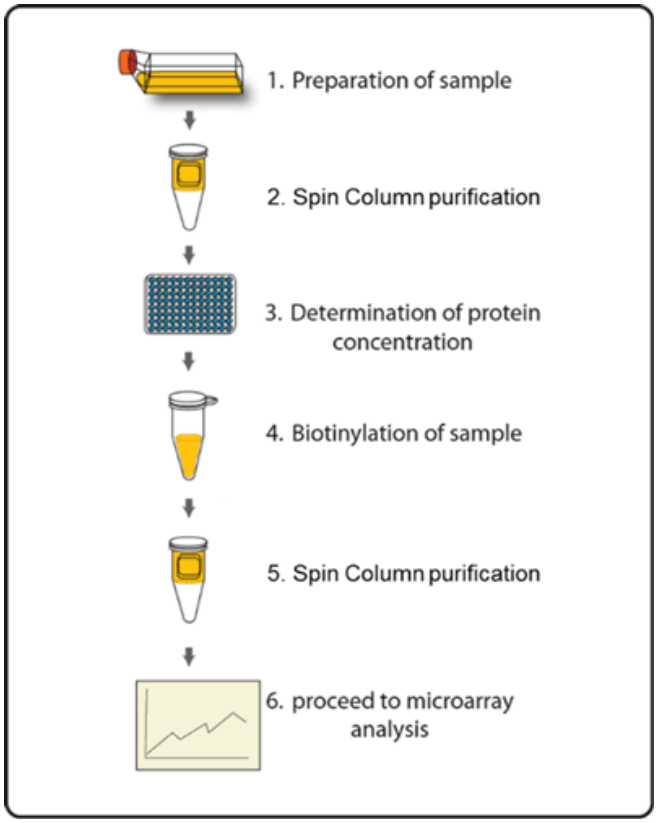
D. Incubations and Washes

- 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.

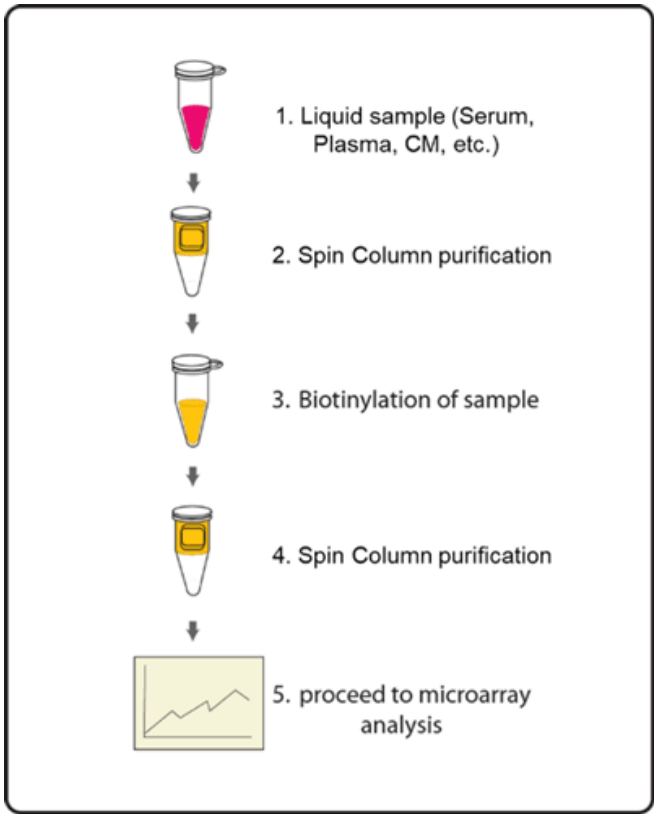
IV. Protocol

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.

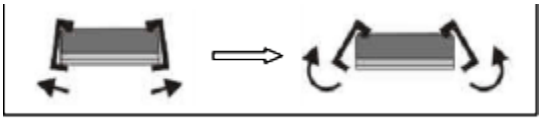
    Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

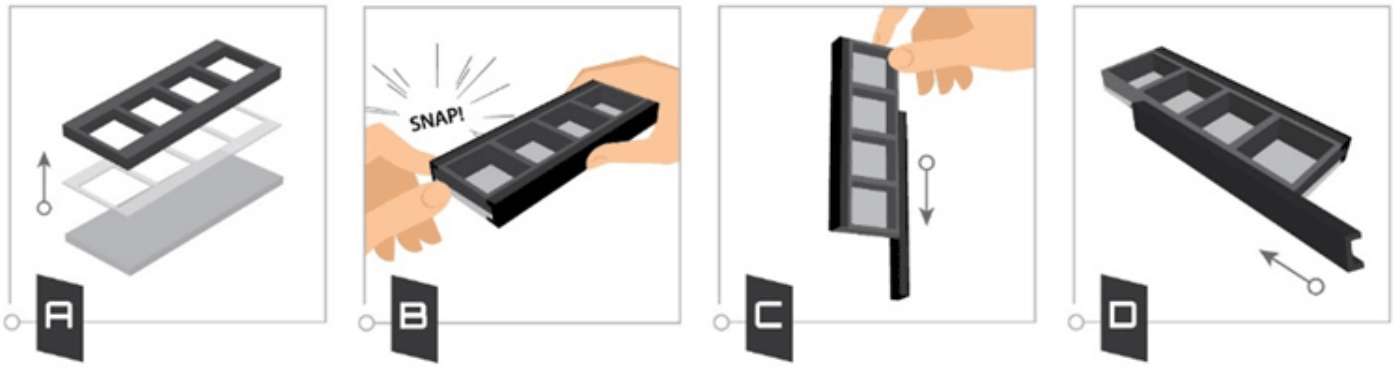
E. Fluorescence Detection

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	ABL1	73	CDK4	145	FGF19	217	IGF2	289	MMP9	361	Prostasin	433	SULT2A1
2	ACTN1	74	CDK6	146	FGF2	218	IGFBP2	290	MPO	362	PSA	434	SYK
3	AFP	75	CDKN1A	147	FGF20	219	IGFBP3	291	MRAS	363	PTCH1	435	TCF3
4	AKT1	76	CDKN1B	148	FGF21	220	IKBKB	292	MSH2	364	PTCH2	436	TCF7
5	AKT2	77	CDKN2A	149	FGF22	221	IKBKG	293	MSLN	365	PTEN	437	TCF7L1
6	AKT3	78	CDKN2B	150	FGF23	222	IL12B	294	MYC	366	PTGS2	438	TGFA
7	ALDH3A1	79	CEA	151	FGF3	223	IL1R2	295	NANOG	367	PTK2	439	TGFB1
8	ALK	80	CEBPA	152	FGF4	224	IL-2	296	NCOA3	368	PTPN11	440	TGFB2
9	APC	81	CEBPE	153	FGF5	225	IL2RB	297	NCOR1	369	PTPN6	441	TGFB3
10	ARAF	82	CHGA	154	FGF6	226	IL3	298	NFKB1	370	PXN	442	TGFBR1
11	ARHGEF1	83	CHUK	155	FGF7	227	IL6ST	299	NFKB2	371	RAC1	443	TGFBR2
12	ARHGEF6	84	CKMB	156	FGF8	228	IL-8	300	NFKBIA	372	RAC3	444	THBS1
13	ARNT	85	COL1A1	157	FGF9	229	ILK	301	NGFR	373	RAD51	445	TIMP1
14	ATF1	86	COL4A1	158	FGFR1	230	INSRR	302	NKX3-1	374	RAF1	446	TIMP3
15	ATF2	87	CREB1	159	FGFR2	231	IRF3	303	NOS2	375	RALA	447	TLR2
16	ATF4	88	CREB3L1	160	FGFR3	232	IRS1	304	NOTCH1	376	RALB	448	TLR4
17	ATM	89	CREB3L2	161	FHIT	233	ITGA2	305	NOTCH2	377	RANK	449	TMPRSS2
18	AXIN1	90	CREBBP	162	FLT1	234	ITGA3	306	NOTCH3	378	RAP1A	450	TNC
19	AXIN2	91	CRK	163	FLT3	235	ITGA5	307	NOTCH4	379	RARA	451	TNFSF11
20	B2M	92	CRKL	164	FLT4	236	ITGA6	308	NR4A3	380	RARB	452	TNR
21	B7-H4	93	CSF1R	165	FN1	237	ITGAM	309	NRAS	381	RASSF1	453	TNXB
22	BAD	94	CSF2	166	FOS	238	ITGAV	310	NSE	382	RB1	454	TP53
23	BAK1	95	CTNNB1	167	FOXO1	239	ITGB1	311	NTRK1	383	RBPJ	455	TP63
24	BAX	96	CYCS	168	FOXP1	240	ITGB3	312	NTRK3	384	RECK	456	TRADD
25	BCL2	97	CYFRA21-1	169	FUT8	241	ITGB5	313	OPN	385	REL	457	TRAF1
26	BCL2L1	98	CYP1B1	170	FZD1	242	ITGB7	314	PAK1	386	RELA	458	TRAF2
27	BCL2L11	99	DDB2	171	FZD2	243	JAG1	315	PAK2	387	RET	459	TRAF3
28	BCL6	100	DDX5	172	FZD3	244	JAG2	316	PAK3	388	RHEB	460	TRAF4
29	BCR	101	DGKB	173	FZD4	245	JAK1	317	PAK4	389	RHOA	461	TRAF5
30	BIRC2	102	DGKD	174	FZD5	246	JAK3	318	PAK6	390	ROCK1	462	TRAF6
31	BIRC3	103	DGKE	175	FZD6	247	JUN	319	PAX3	391	ROCK2	463	TRIM71
32	BMI1	104	DGKG	176	FZD7	248	JUP	320	PAX5	392	RPS6	464	TSC1
33	BMP2	105	DGKI	177	FZD8	249	KDR	321	PAX7	393	RPS6KA5	465	TSC2
34	BMP4	106	DGKQ	178	GADD45A	250	KIAA1303	322	PAX8	394	RPS6KB1	466	TWIST1
35	BMPR2	107	DGKZ	179	GADD45B	251	KLK10	323	PDCD4	395	RRAS2	467	TWIST2
36	BRAF	108	DLL1	180	GADD45G	252	KLK6	324	PDGFA	396	RUNX1	468	TYMP
37	BRCA1	109	DLL3	181	GCK	253	KRAS	325	PDGFB	397	RUNX2	469	UGT1A1
38	BRCA2	110	DLL4	182	GLI1	254	LAMA1	326	PDGFC	398	RXRA	470	VAV1
39	C3	111	DUSP6	183	GLI2	255	LAMA3	327	PDGFD	399	RXRB	471	VEGFA
40	CA125	112	DVL1	184	GLI3	256	LAMA4	328	PDGFRA	400	RXRG	472	WAS
41	CA15-3	113	DVL2	185	GP73	257	LDHA	329	PDGFRB	401	SCCA1	473	WASF1
42	CA19-9	114	DVL3	186	GPC1	258	LDHB	330	PDPK1	402	SCF	474	WASF3
43	CA242	115	E2F1	187	GPC3	259	LEF1	331	PFKM	403	SDC1	475	WNT1
44	CA72-4	116	E2F2	188	GRB2	260	Leptin	332	PGA3	404	SDC2	476	WNT10A
45	CAMK2A	117	EFNA3	189	GSK3B	261	LMO2	333	PGR	405	SDC4	477	WNT10B
46	CAMK2B	118	EFNA4	190	GSN	262	LRP5	334	PIK3CA	406	SERPINB5	478	WNT11
47	CAMK2D	119	EFNA5	191	GSTP1	263	LRP6	335	PIK3CB	407	SHC1	479	WNT16
48	CASP3	120	EGF	192	GZMB	264	LTBR	336	PIK3CD	408	SHC2	480	WNT2
49	CASP8	121	EGFR	193	HBEGF	265	LUM	337	PIK3R1	409	SHH	481	WNT2B
50	CASP9	122	EGLN1	194	HCGb	266	MAD1L1	338	PIK3R2	410	SIN3A	482	WNT3A
51	CAV1	123	EGLN2	195	HDAC1	267	MAF	339	PIK3R3	411	SIRT1	483	WNT4
52	CAV2	124	EGLN3	196	HDAC2	268	MAP2K1	340	PIM1	412	SIRT3	484	WNT5A
53	CAV3	125	EIF2AK2	197	HDAC4	269	MAP2K2	341	PIM2	413	SKP2	485	WNT5B
54	CBL	126	EIF4B	198	HE4	270	MAPK1	342	PLA2G4A	414	SLC22A1	486	WNT6
55	CCNA1	127	EIF4EBP1	199	HGF	271	MAPK11	343	PLAT	415	SLC22A2	487	WNT7A
56	CCNA2	128	ELK1	200	HHEX	272	MAPK12	344	PLAU	416	SLC2A1	488	WNT7B
57	CCND1	129	EP300	201	HIF1A	273	MAPK3	345	PLAUR	417	SLC2A2	489	WNT8B
58	CCND2	130	EPAS1	202	HIST3H3	274	MAPK7	346	PLCG1	418	SLC7A5	490	WNT9A
59	CCND3	131	ERBB2	203	HK2	275	MAPK8	347	PLCG2	419	SMAD2	491	WNT9B
60	CCNE1	132	ERBB3	204	HMGA2	276	MAPK9	348	PLD1	420	SMAD3	492	YWHAB
61	CCNE2	133	ERBB4	205	HMOX1	277	MARCKS	349	PLD2	421	SMAD4	493	YWHAE
62	CCR3	134	EREG	206	HOXD10	278	MAX	350	PML	422	SMO	494	YWHAH
63	CCR5	135	ESR1	207	HPGD	279	MCL1	351	PPARD	423	SOS2	495	YWHAQ
64	CCR8	136	ESR2	208	HPN	280	MCSF	352	PPARG	424	SP1	496	YWHAZ
65	CD14	137	ETV5	209	HRAS	281	MDM2	353	PPP1CA	425	SPI1	497	ZBTB16
66	CD44	138	EZR	210	HSD11B1	282	MEF2C	354	PRKACA	426	SPINT1	498	ZBTB17
67	CDC25A	139	FCGR1A	211	HSP90AB1	283	MEN1	355	PRKACB	427	SPRY2	499	ZEB1
68	CDC25B	140	Ferritin	212	HSP90B1	284	MET	356	PRKACG	428	SRC	500	ZEB2
69	CDC25C	141	FGF1	213	HSPG2	285	MITF	357	PRKCA	429	ST14		
70	CDC42	142	FGF10	214	ID2	286	MMP16	358	PRKCB	430	STAT3		
71	CDH1	143	FGF16	215	IGF1	287	MMP2	359	PRKCE	431	STAT5A		
72	CDK2	144	FGF17	216	IGF1R	288	MMP3	360	PRKCG	432	STAT5B		

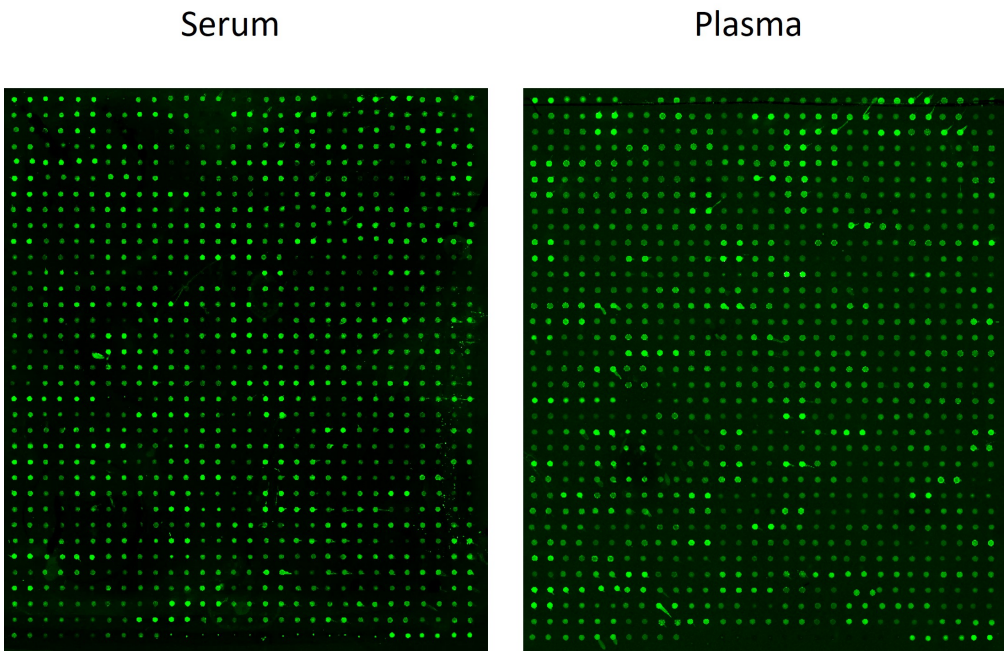
VII. Interpretation of Results

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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