RayBio® Label-Based (L-Series) Human L10 Array, Glass Slide

Patent Pending Technology
User Manual (Jan 1, 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-10-4 (4 Sample Kit)
AAH-BLG-10-8 (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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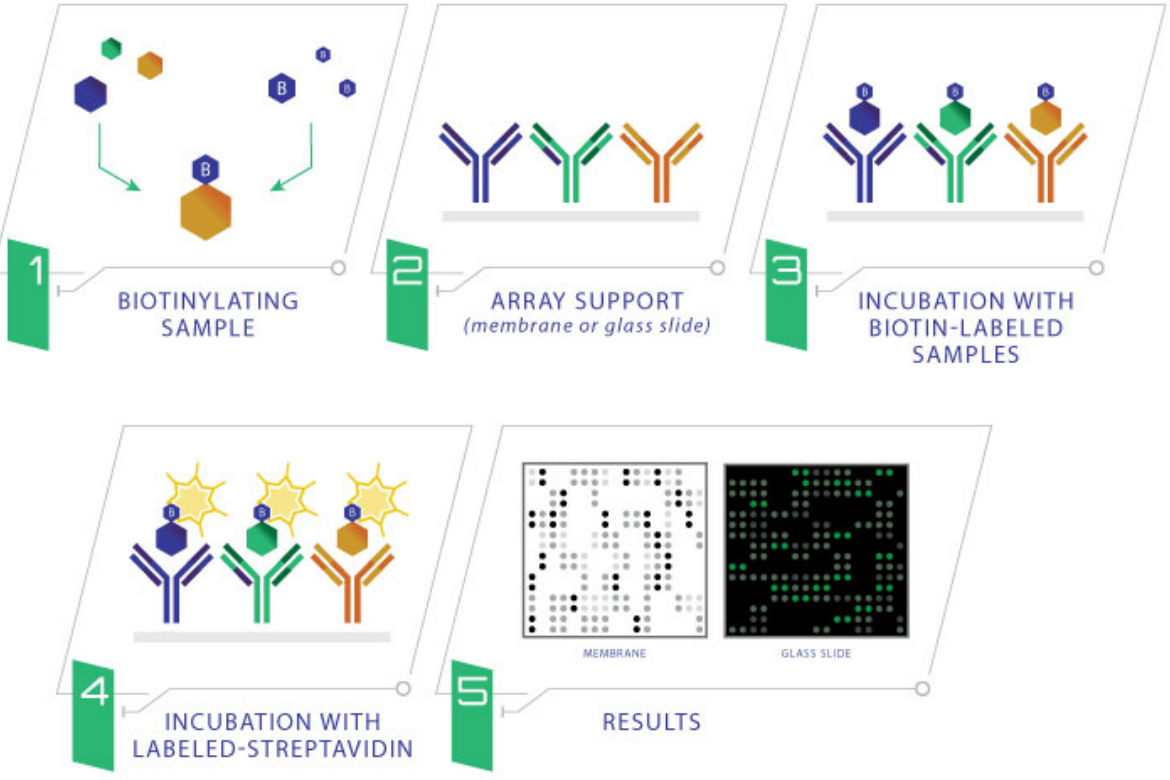
I. Introduction

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.

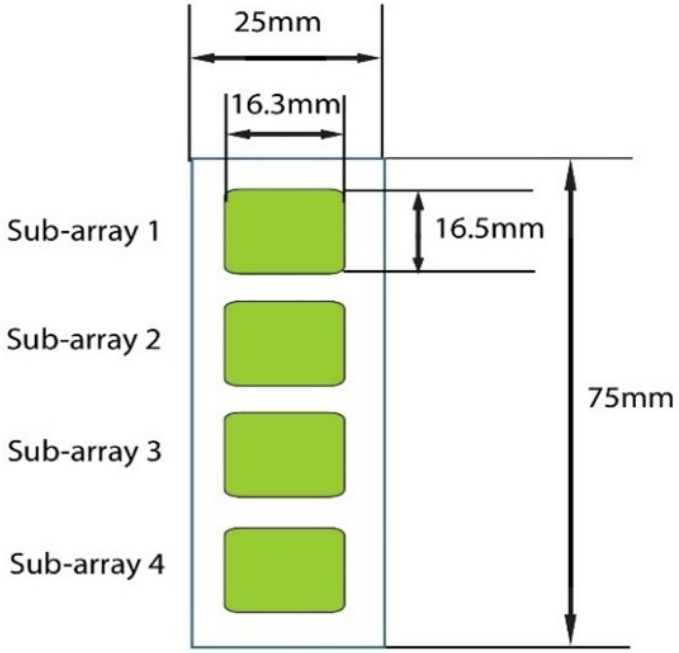
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

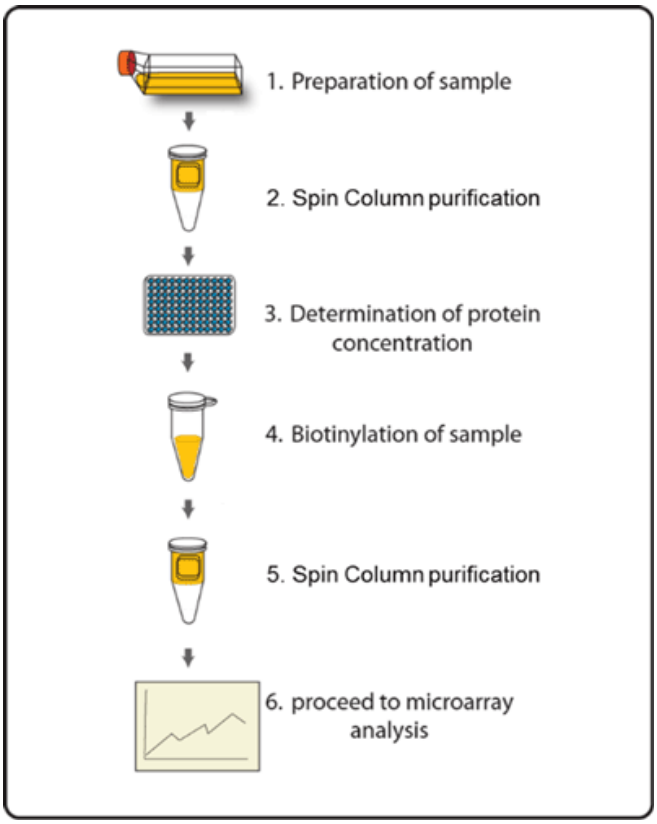
D. Incubations and Washes

- 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.

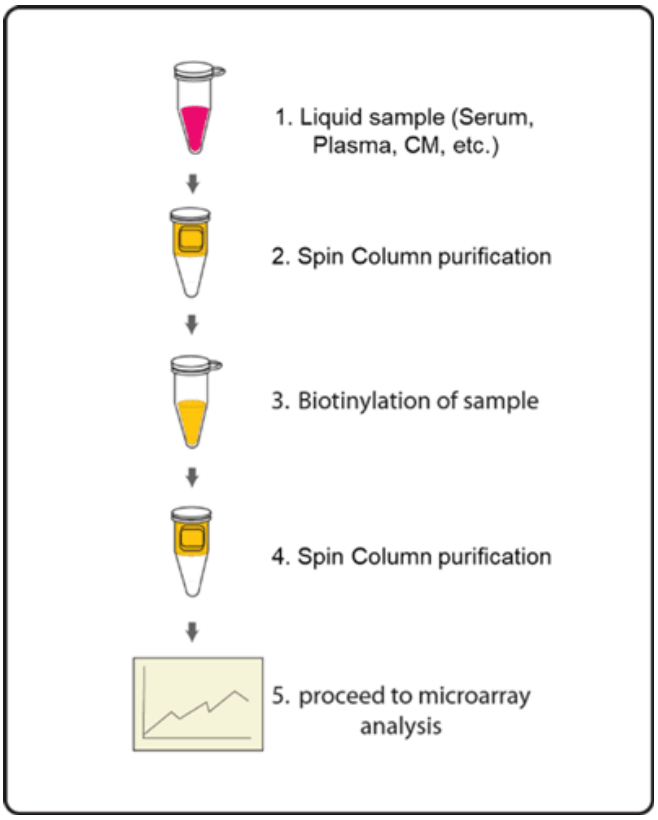
IV. Protocol

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 <u>not</u> 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 <u>completely</u> 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.

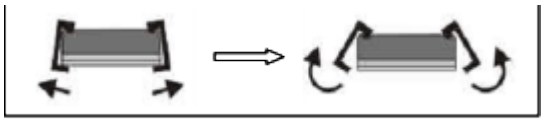
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

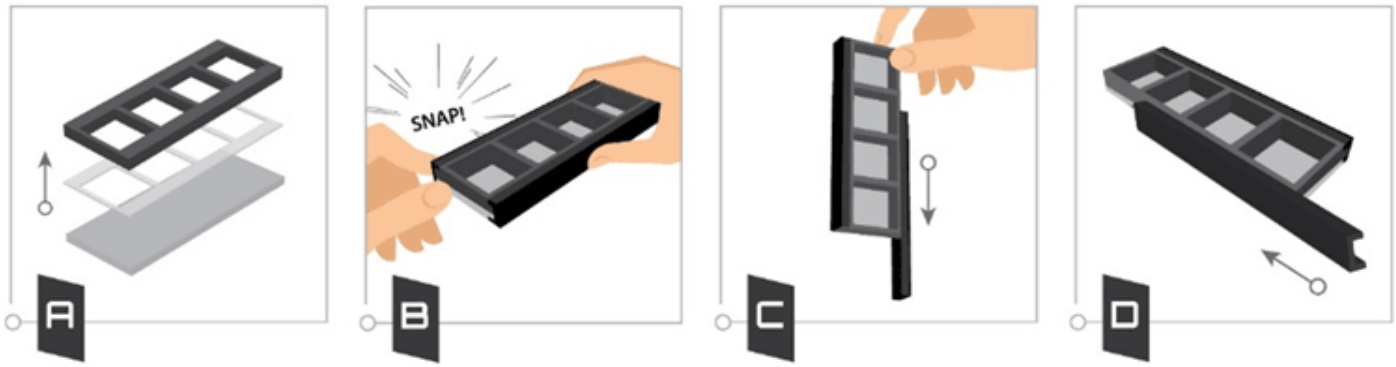
E. Fluorescence Detection

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: <u>Please protect the finished glass slides from temperatures above RT and store them in the dark.</u> 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	A4GALT	73	CASQ2	145	EIF3D	217	IGSF8	289	OLFM4	361	PXMP4	433	SSR1
2	ABCB9	74	CBFB	146	EIF3G	218	IMPA1	290	ORAI3	362	RAB11B	434	STAG1
3	ABCC2	75	CBLC	147	EIF3J	219	INSL5	291	OS9	363	RAB27B	435	STC2
4	ABCC3	76	CBR3	148	EIF3K	220	ISM1	292	OTX1	364	RAB31	436	STK16
5	ABCD1	77	CD247	149	EIF4EBP1	221	ITCH	293	OXCT1	365	RAB6A	437	STX8
6	ABHD14B	78	CD3E	150	EIF5A2	222	ITGAE	294	P2RX5	366	RAB8B	438	SYT6
7	ABI2	79	CD81	151	ELN	223	KCNJ12	295	P2RY6	367	RAD54B	439	TAZ
8	ABLIM1	80	CD8B	152	EMR1	224	KCNQ5	296	PABPC3	368	RAET1L	440	TBCB
9	ACADL	81	CDC37	153	EPN3	225	KIAA0101	297	PADI4	369	RBKS	441	TBP
10	ACIN1	82	CDC42	154	ERCC5	226	KIAA1279	298	PAPOLA	370	RBL2	442	TFDP1
11	ACOT13	83	CDC42BPB	155	EWSR1	227	KLF13	299	PARD6B	371	RBM17	443	TJP1
12	ACOT2	84	CDC6	156	EXOSC10	228	KLF7	300	PARVA	372	RBM34	444	TJP2
13	ACOX1	85	CDCA3	157	F8	229	KLRC1	301	PCF11	373	RBP5	445	TLE3
14	ACSBG1	86	CDH16	158	FAM98A	230	KRT24	302	PDCD10	374	RBP7	446	TLK1
15	ACSL1	87	CDK6	159	FAR2	231	KRT7	303	PDCD6IP	375	RELA	447	TP53I3
16	ACSL5	88	CDS2	160	FAU	232	KRT78	304	PDE1C	376	REXO1	448	TPPP3
17	ACSS3	89	CEBPB	161	FBL	233	KRT79	305	PDE9A	377	RFK	449	TPT1
18	ACYP2	90	CGREF1	162	FBXO6	234	LAPTM4A	306	PDK1	378	RHOT1	450	TRDMT1
19	ADCYAP1R1	91	CHMP4A	163	FGFBP3	235	LIG1	307	PDRG1	379	RING1	451	TRH
20	ADK	92	CHN1	164	FHIT	236	LIN52	308	PDYN	380	RIOK2	452	TSG101
21	Adracalin	93	CHRNA7	165	FSTL5	237	LRIG1	309	PEX13	381	RNASEH1	453	TSPAN1
22	ADSL	94	CKMT1A	166	GABARAPL2	238	LRP10	310	PEX19	382	RNASEH2B	454	TST
23	AIM2	95	CLCA1	167	GADD45G	239	LRP11	311	PEX3	383	RNF41	455	TTF1
24	AKR1A1	96	CLDN7	168	GALE	240	LRP12	312	PFDN5	384	RNMT	456	TTF2
25	AKR1B10	97	CLPB	169	GALK1	241	LSM3	313	PFKFB4	385	RPL36	457	TUBG1
26	ALOX15B	98	CMBL	170	GALNS	242	LSM7	314	PFKP	386	RPP14	458	TXNL4A
27	ALOX5AP	99	CNOT6	171	GBP2	243	LSP1	315	PFN2	387	RPRD1B	459	TXNRD1
28	AMFR	100	CNPY2	172	GCDH	244	LTC4S	316	PGA4	388	RRAGC	460	TYMS
29	ANAPC13	101	COASY	173	GCM2	245	MAG	317	PGS1	389	RUVBL1	461	TYRP1
30	ANO1	102	COL6A3	174	GFER	246	MAGOH	318	PHB2	390	RXFP3	462	UBASH3A
31	AP1B1	103	COMMD8	175	GGA1	247	MAN1A2	319	PHF1	391	SACM1L	463	UBE2F
32	AQP1	104	CPLX3	176	GGA3	248	MAP4K3	320	PHF13	392	SCARB1	464	UBE2H
33	AQP9	105	CPSF6	177	GLIPR1	249	MAPK1	321	PIAS1	393	SCLY	465	UBE2I
34	ARD1A	106	CPT2	178	GLT8D2	250	MAPK8	322	PIGK	394	SCN2B	466	UBE2M
35	ARF3	107	CPVL	179	GMEB1	251	MAPKAPK3	323	PIK3CA	395	SCPEP1	467	UBE2T
36	ARID1A	108	CRABP1	180	GMFG	252	MAPRE3	324	PIM1	396	SDF2	468	UBE2W
37	ARID1B	109	CRABP2	181	GNG13	253	MASP2	325	PIPOX	397	SDHB	469	UBE3A
38	ARL2BP	110	CRADD	182	GNGT1	254	MAX	326	PLBD2	398	SEC11C	470	UBQLN2
39	ASB2	111	CRELD1	183	GOPC	255	MCM8	327	PLK4	399	SEC23B	471	UBXN6
40	ASF1A	112	CRYZ	184	GPD2	256	MCTS1	328	PLN	400	SECTM1	472	UGDH
41	ATIC	113	CSNK1G2	185	GPR37	257	MECP2	329	PLSCR3	401	SELM	473	UGP2
42	ATL3	114	CSNK2A2	186	GRIK2	258	MRPL11	330	PMCH	402	SETD8	474	UNC13C
43	ATOX1	115	CST7	187	GSTK1	259	MRPL12	331	PML	403	SF3B4	475	UNC5A
44	ATP1B1	116	CTSK	188	GSTM5	260	MRPL15	332	PMM2	404	SHOC2	476	UNG
45	ATP6V0A2	117	CUTC	189	GSTZ1	261	MRPL30	333	PNPT1	405	SIPA1	477	UPF3B
46	ATP6V1F	118	CYP19A1	190	GUCA1A	262	MRPL44	334	POLR3B	406	SIRT4	478	UROS
47	ATPIF1	119	DAO	191	HAT1	263	MRPS2	335	POP4	407	SLC19A3	479	USP30
48	AZI2	120	DCLK1	192	HDAC1	264	MRPS5	336	PPIE	408	SLC22A7	480	USP46
49	B3GAT3	121	DCUN1D1	193	HDAC3	265	MRTO4	337	PPIF	409	SLC22A8	481	UTP18
50	B3GNT3	122	DDX41	194	HDAC6	266	MS4A1	338	PPIP5K2	410	SLC26A6	482	UXS1
51	BABAM2	123	DHRS2	195	HERC2	267	MTSS1	339	PPP1CC	411	SLC27A4	483	VARS
52	BCL2L1	124	DHRS4	196	HERPUD1	268	MUS81	340	PPP1R17	412	SLC2A10	484	VKORC1
53	BCL2L12	125	DHRS9	197	HIST1H2BB	269	MVP	341	PPP2R5E	413	SLC34A1	485	VLDLR
54	BCL6	126	DHX15	198	HIST1H3A	270	NAALADL1	342	PPT1	414	SLC34A3	486	VNN2
55	BDKRB1	127	DIS3L	199	HIST2H2BE	271	NANOS1	343	PQBP1	415	SLC39A8	487	VPS25
56	BET1L	128	DNAJC30	200	HNF1A	272	NARS	344	PRAC1	416	SLC6A1	488	VPS26A
57	BHLHE40	129	DNASE2	201	HNMT	273	NCKAP1	345	PRAP1	417	SLC9A3R1	489	VRK1
58	BLNK	130	DNM1	202	HRAS	274	NCL	346	PRDM2	418	SLK	490	VSNL1
59	BMF	131	DNTT	203	HSD3B1	275	NDRG1	347	PRICKLE2	419	SMG1	491	VTA1
60	BOLA1	132	DPF3	204	HSPA6	276	NFE2L3	348	PRKAR1A	420	SMN1	492	VTI1A
61	BPHL	133	DPYSL5	205	HTR3B	277	NGLY1	349	PRKD2	421	SMPD2	493	VTI1B
62	BTG3	134	DUSP14	206	HTRA3	278	NMRAL1	350	PRKG2	422	SMYD2	494	WDR61
63	BTN3A3	135	DZIP1	207	IDI2	279	NOS1AP	351	PRMT5	423	SNRPD2	495	WWC1
64	BVES	136	E2F5	208	IDO2	280	NPC1	352	PRMT6	424	SNX2	496	XCL2
65	CA7	137	EBPL	209	IFITM2	281	NSFL1C	353	PRPF18	425	SP1	497	XPC
66	CALM2	138	ECD	210	IFNA10	282	NTNG1	354	PRPF19	426	SP100	498	XRCC1
67	CAMK1	139	ECH1	211	IFNA2	283	NTS	355	PSME1	427	SP4	499	XRCC2
68	CAMK4	140	ECSIT	212	IFNA5	284	NUDC	356	PSME2	428	SPCS2	500	ZFP36L2
69	CAPZA2	141	EEF1B2	213	IFNA8	285	NUDT1	357	PSME3	429	SPDYC		
70	CAPZB	142	EFNA1	214	IFNW1	286	NUP107	358	PSMF1	430	SPEG		
71	CASP5	143	EHD2	215	IGF2BP3	287	NUP50	359	PTGES	431	SRP72		
72	CASP6	144	EIF1AY	216	IGJ	288	NUP62	360	PTPN12	432	SRSF10		

VII. Interpretation of Results:

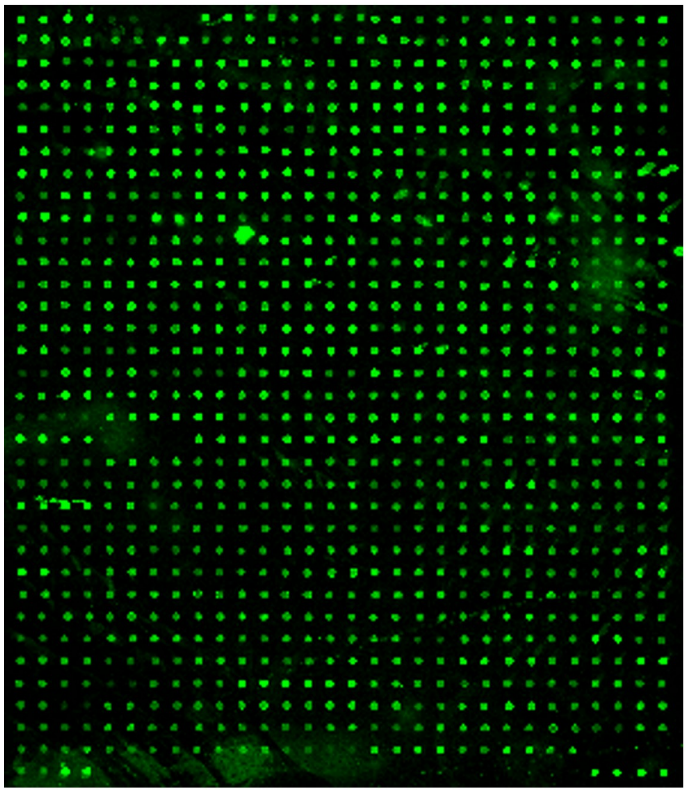
A. Explanation of Controls Spots

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.

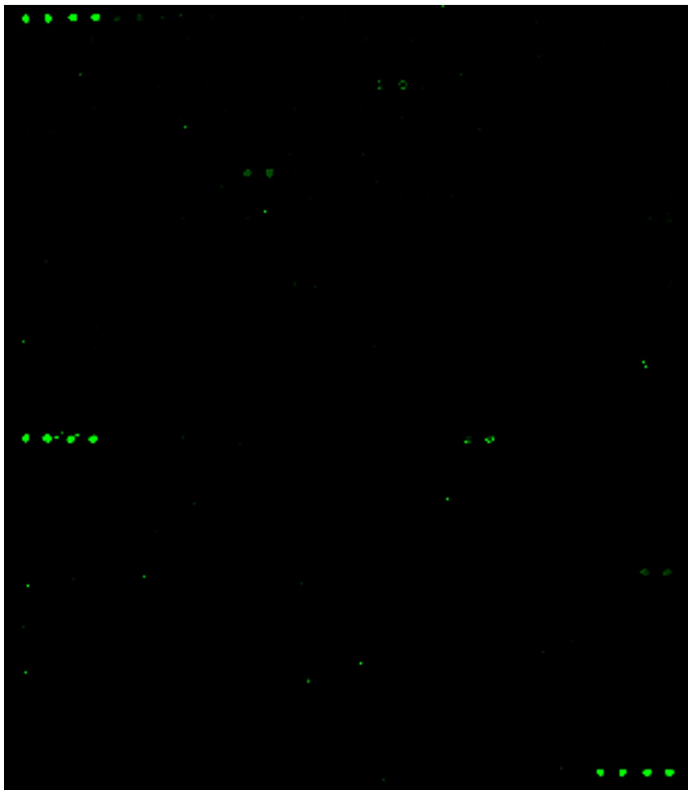
B. Typical Results

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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