RayBio® Label-Based (L-Series) Human L4 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-4-4 (4 Sample Kit)
AAH-BLG-4-8 (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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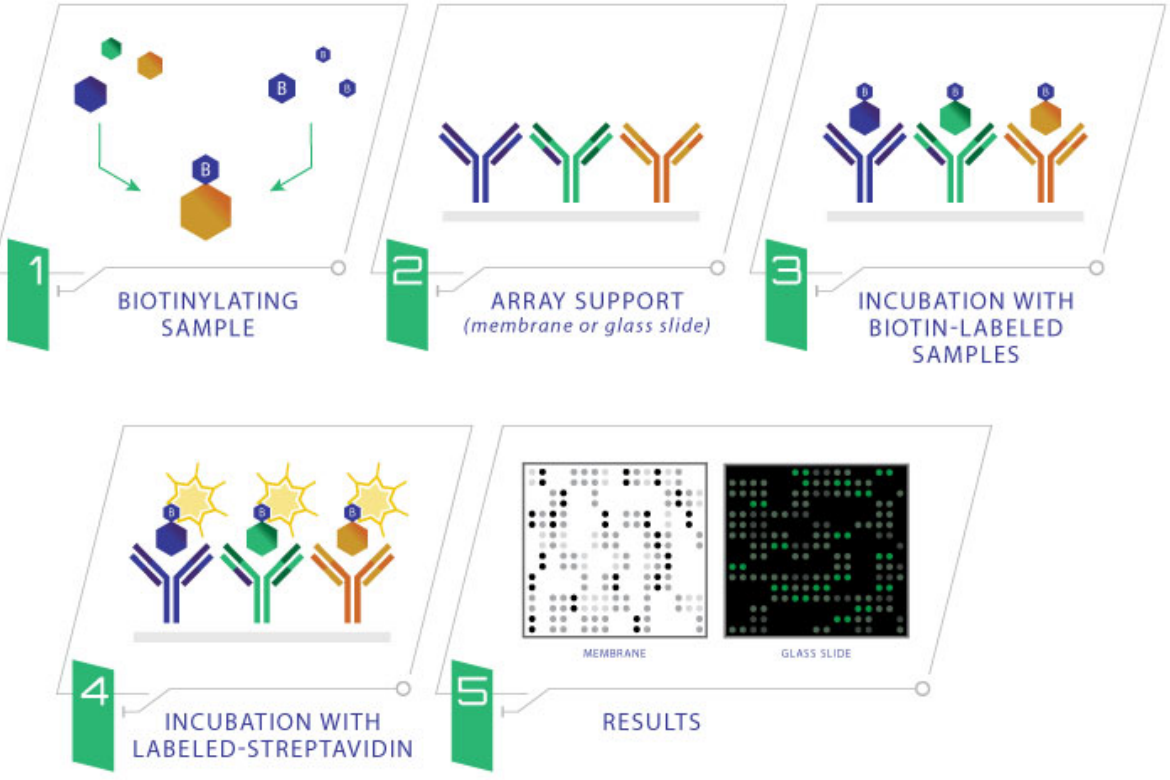
I. Introduction

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.

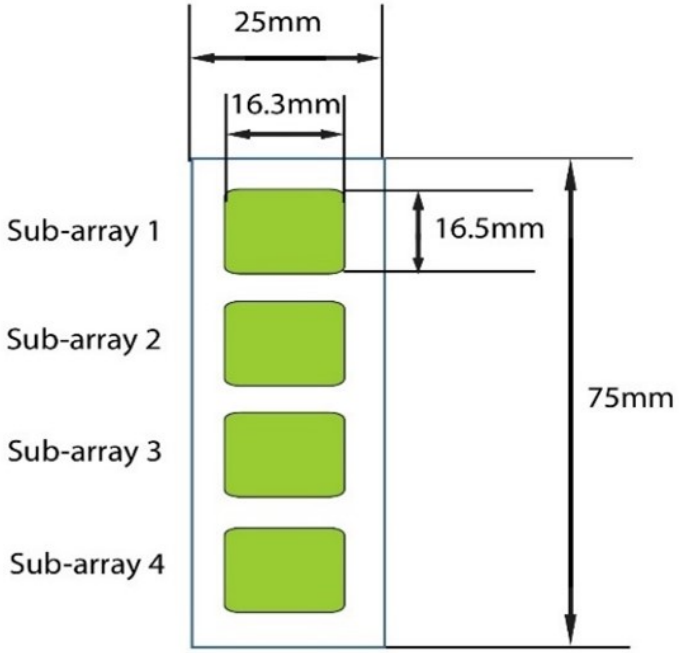
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

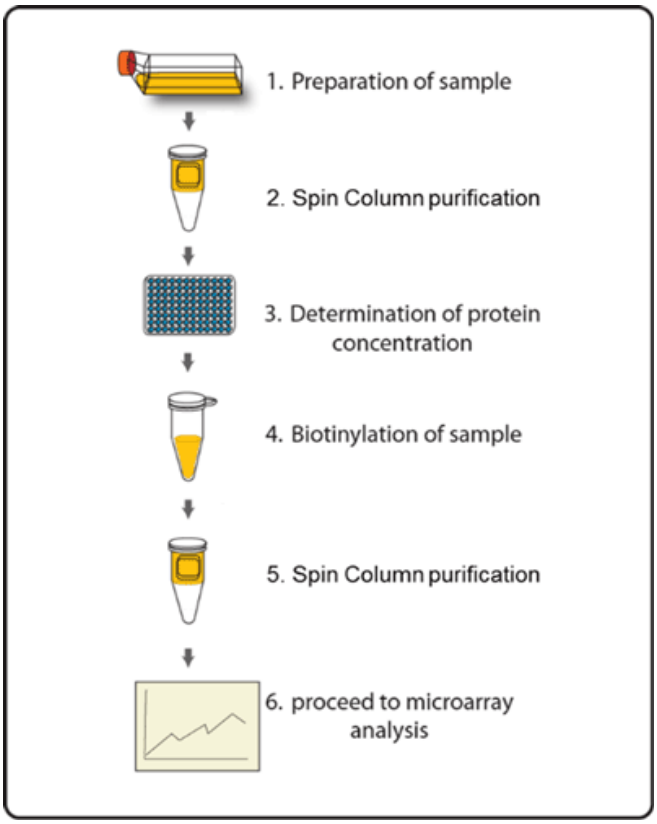
D. Incubations and Washes

- 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.

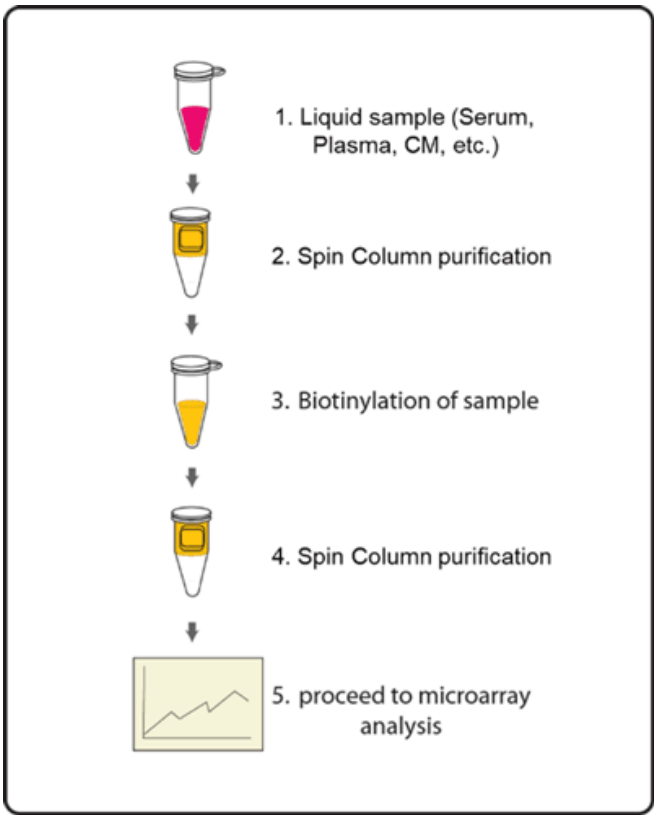
IV. Protocol

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.

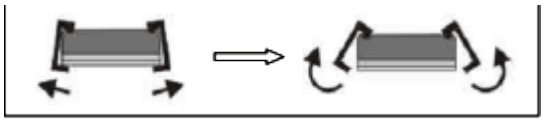
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

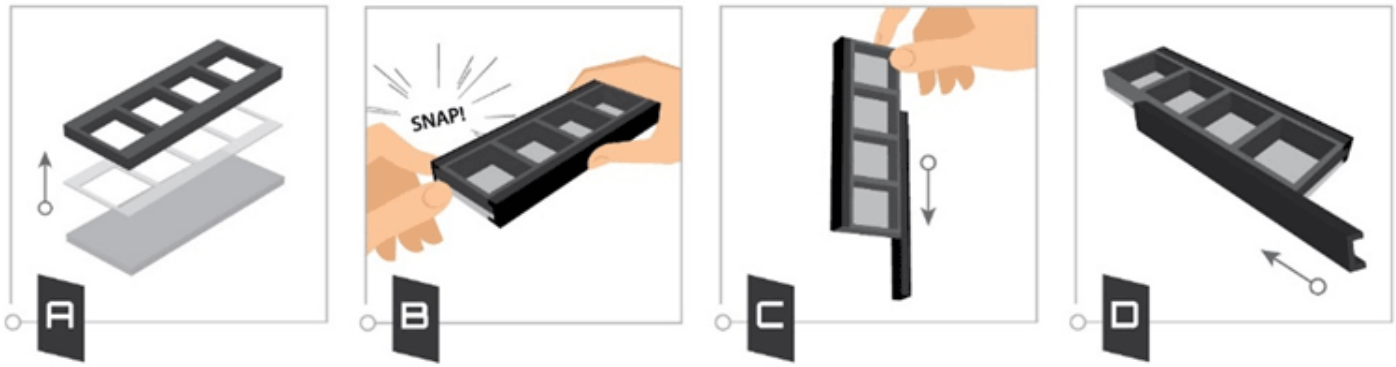
E. Fluorescence Detection

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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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
2	12	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25	25	26	26
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35	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	POS3	POS3	POS2	POS2	POS1	POS1

VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	HEXA	73	LIMS1	145	Nectin-1	217	Peroxiredoxin 3	289	PTK 7	361	Serpin A7	433	Thymosin b10
2	HTRA1	74	LMAN2	146	Nectin-3	218	Peroxiredoxin 5	290	PTMA	362	Serpin B3	434	Titin
3	Agrin	75	ACP1	147	NEDD8	219	PF4V1	291	PTP gamma	363	Serpin B6	435	TLS
4	IBP160	76	LOK	148	Neogenin	220	PGAM1	292	PTP kappa	364	Serpin B8	436	TMEM223
5	IDH1	77	LOX	149	Nesprin2	221	PGAM2	293	PTP mu	365	Serpin F2	437	TOB2
6	IDH3A	78	LOXL1	150	Neurabin 1	222	PGD	294	PTPRS	366	Serpin A10	438	TOP2B
7	IFRD1	79	LRP 4	151	Neural Cadherin	223	PHGDH	295	PTPRZ	367	SERPINB1	439	TPM4
8	IGF2BP2	80	LTA4H	152	PAM	224	PGK-1	296	PYGL	368	SerpinB4	440	TPP1
9	ITGB5	81	LTBP4	153	Neurogranin	225	PGLS-C-t	297	PZP	369	SerpinE2	441	TALDO1
10	IGSF4B	82	Lubricin	154	Neuropeptide B	226	PGM1	298	QDPR	370	SerRS	442	TALDO
11	Ihh	83	LUZP1	155	Neuropilin-1	227	PGRPL	299	QPRT	371	SET	443	Transthyretin
12	ILK	84	LYPA1	156	Neurotrimin	228	PHAP1	300	Quiescin Q6	372	SEZ6L2	444	TRAP1
13	Inhibin beta	85	Lysozyme	157	NF-M	229	PSAT1	301	Rab7a	373	SF20	445	TRAP220
14	ITGB1	86	MAGI2	158	Nidogen-2	230	PIK3C2B	302	Ran	374	SH3BGRL	446	TRF 2
15	ITGB6	87	MAGP-2	159	NIT2	231	pIgR	303	RanGAP1	375	SH3BGRL3	447	TPIS
16	ITGA6	88	MAN1	160	NME3	232	PIK3IP1	304	RAP1AB	376	SHANK1	448	Tropomyosin 3
17	IQGAP1	89	MANF	161	nNOS	233	PIN	305	Rbm15	377	SHC1	449	Twist-1
18	IQGAP2	90	Mannosidase II	162	Noelin	234	PISD	306	RCL	378	SHIP	450	TRPS1
19	IRE1	91	MAP1A	163	Non-muscle Actin	235	PKLR	307	Reg1A	379	SHMT1	451	Trypsinogen-2
20	IRS2	92	MAPRE1	164	Myosin IIA	236	PLA2G1B	308	Reg3A	380	SHP-1	452	Trypsin Pan
21	ISOC2	93	MARCKS	165	Notch-2	237	Plakophilin 1	309	RHOC	381	Siglec-1	453	WRS
22	ITGB4BP	94	MASP3	166	Notch-2 ICD	238	Plastin L	310	RhoGDI	382	SIGLEC14	454	TSR2
23	ITIH1	95	MBD2	167	NPAS3	239	PLC-gamma 1	311	RNASE1	383	SIM2	455	TUBA6
24	ITIH2	96	MBP	168	NPM1	240	Pleckstrin	312	RNH1	384	SIRP beta 1	456	TWF2
25	ITIH3	97	MCAM	169	NQO2	241	Plectin	313	RNASET2	385	Six3	457	TXNDC4
26	ITIH4 a	98	Mcl-1	170	NT5C3	242	Plexin B1	314	RKIP	386	SLC38A10	458	TXNDC5
27	JAM-A	99	MCM	171	NUCB1	243	Plexin B2	315	POLR2A	387	SLITRK1	459	TXNRD2
28	JARID2	100	MCM5	172	NUP98	244	PLOD1	316	RNASE4	388	SLURP1	460	UBE2D3
29	KPNB1	101	MCMP2	173	OBCAM	245	PLOD2	317	RNASE6	389	SMA	461	Ube2L3
30	Keratin 36	102	MDH1	174	OIT3	246	PLS3	318	RPL10	390	SMC4	462	UBE2N
31	Keratin 38	103	MDH2	175	Olfactomedin-2	247	Plxdc2	319	RPL10A	391	SMPD4	463	Ubiquitin
32	KHSRP	104	ME1	176	OTC	248	PNP	320	RPL11	392	SOD1	464	UCH-L1
33	KIAA0319L	105	MEP1A	177	Orosomucoid 2	249	POR	321	RPL12	393	SOD2	465	UFM 1
34	KIAA1468	106	Metallothionein	178	ORP150	250	PPCS	322	RPL14	394	SOD-3	466	UGGT
35	KIAA1967	107	Metavinculin	179	OSBP1	251	PPOX	323	RPL17	395	SOD4	467	UNC13D
36	KIF5B	108	MFAP4	180	OSCAR	252	PPP2R1B	324	RPL22	396	Somatostatin	468	UNC45A
37	Kilon	109	MFI2	181	OSM R beta	253	PPP2R4	325	RPL5	397	SORD	469	UNC5H4
38	KLK-B1	110	mGLUR5	182	Osteoadherin	254	PRCP	326	RPL7A	398	SorLA	470	UPB1
39	KMD4B	111	MGP	183	OXT	255	PRDM13	327	RPLP0	399	SOX4	471	UQCRB
40	KMT2B	112	Mimecan	184	p16 ARC	256	PRDX 1	328	RPS10	400	SP-D	472	UQCRH
41	KRT31	113	MINPP1	185	P20Sb3	257	PRELP	329	RPS11	401	Spectrin beta-5	473	URB
42	KRT72	114	MLCK	186	p23	258	PREP	330	RPS12	402	SPEN	474	URB2
43	Krt73	115	MMR	187	p39	259	PRG2	331	RPS19	403	SPINK7	475	UROC1
44	KRT82	116	MMRN1	188	P4HB	260	PRNP	332	RPS2	404	SPTBN1	476	UROD
45	KRT85	117	MN1	189	p73	261	Profilin 1	333	RPS20	405	Src	477	URP2
46	KRTDAP	118	Moesin	190	PA2G4	262	Properdin	334	RPS23	406	SREC-II	478	USP14
47	KRTHA3B	119	MP1	191	PABP	263	Prosaposin	335	RPS25	407	STAT3	479	USP5
48	KSR1	120	MPCA	192	PACS1	264	PTGDS	336	RPS28	408	Stathmin 1	480	Uteroglobin
49	LAD	121	MPO	193	PARVB	265	PSMB6	337	RPS3	409	SCP2	481	Utrophin
50	LAF4	122	MRP 1	194	PCBP1	266	PSMA3	338	RPS5	410	STI1	482	VAP-1
51	LAIR1	123	MSH6	195	PCBP2	267	PSMA5	339	RREB1	411	STOM	483	VAP-A
52	LAM b1	124	mTOR	196	PCCA	268	PSMB7	340	RSU1	412	SUCLG1	484	VCP
53	LAMA	125	MUCDHL	197	PCDH7	269	PSMD5	341	S100A1	413	SUMO3	485	VDAC1
54	LMNA	126	Multimerin 2	198	PCDX8	270	PSMB1	342	S100A11	414	Symplekin	486	Versican
55	LMNB1	127	MyBPC3	199	PCK2	271	PSMA6	343	S100A7	415	SynCAM	487	Vimentin B
56	LMNB2	128	MYH2	200	PCMT1	272	PSB2	344	S100A9	416	Syntaxin 7	488	VNN1
57	LAMA2	129	MYH6	201	PCNA	273	PSB4	345	S100P	417	TAB182	489	VSIG4
58	LAMB2	130	MYH7	202	PCPE-1	274	Protein C	346	TIM-4	418	TAGLN2	490	WDR1
59	LAMC1	131	MYHC	203	PCSK9	275	Protein Z	347	SAA4a	419	Talin1	491	WISP2
60	LAMP	132	MYL12B	204	PCYOX1	276	Prouroguanylin	348	aAmylase	420	Talin1&2	492	WNK2
61	LAMP1	133	MYL3	205	PDE1B	277	PRSS23	349	SAMSN1	421	TAX1BP3	493	YB1
62	LAMP2	134	MYO5A	206	PDIA6	278	PRSS3	350	SBP-1	422	TBCA	494	YY1
63	LAP3	135	Myoferlin	207	PDLIM1	279	PRTN3	351	SBSN	423	TCEB2	495	ZBTB4
64	LASP1	136	Myosin 18B	208	PDLIM5	280	PSMA1	352	SDF4	424	Tcf20	496	ZC3H4-N-t
65	LTBP2	137	Myotrophin	209	PDZD2	281	PSMA2	353	SDNSF	425	TCN1	497	ZC3H8
66	LCAT	138	NABC1	210	PEBP4	282	PSMA4	354	SDPR	426	TCP1 eta	498	ZDHHC18
67	LCMT2	139	NAGLU	211	PEPD	283	PSMA7	355	SCG5	427	Tenascin C	499	ZNF671
68	LDHA	140	NAP1L1	212	PER1	284	PSMB5	356	Semaphorin 6B	428	Tenascin X	500	Zyxin
69	LDHB	141	NAPRT1	213	perilipin 3	285	PSMC3	357	Semaphorin 7A	429	TFF2		
70	LEDGF	142	NASP	214	Perilipin-1	286	PSMD1	358	SEMG1	430	TGM3		
71	SPINK5	143	NCAM2	215	Periostin	287	PSMD9	359	SEMG2	431	Thioredoxin-1		
72	LILRA3	144	Nebulin	216	Peroxiredoxin 2	288	PTEN	360	Serpin A11	432	THOP1		

VII. Interpretation of Results:

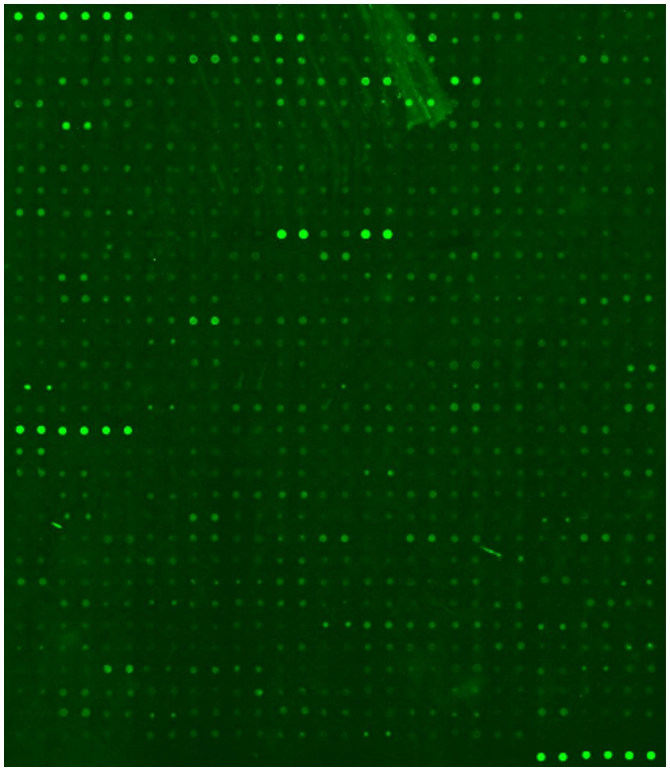
A. Explanation of Controls Spots

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.

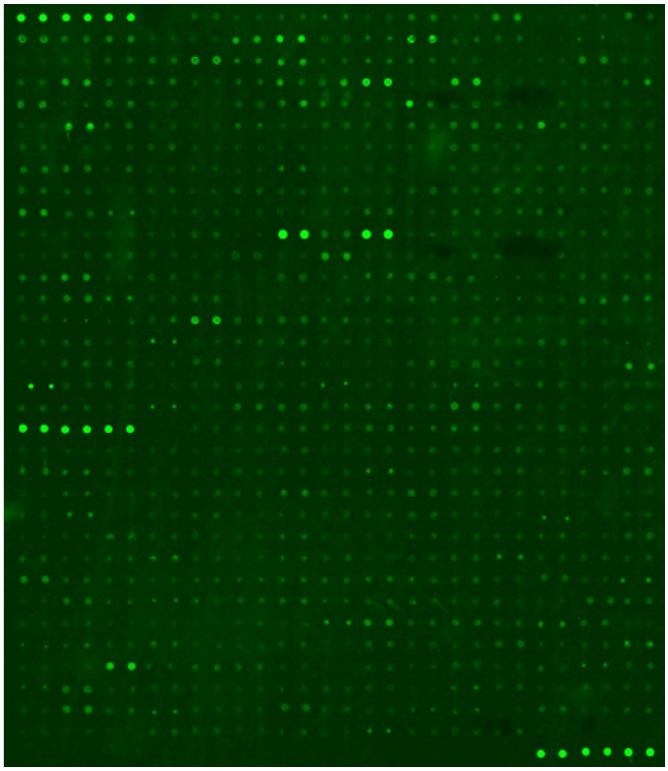
B. Typical Results

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.

Serum



Plasma



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

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