RayBio® Label-Based (L-Series) Mouse L3 Array, Glass Slide

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAM-BLG-3-4 (4 Sample Kit)
AAM-BLG-3-8 (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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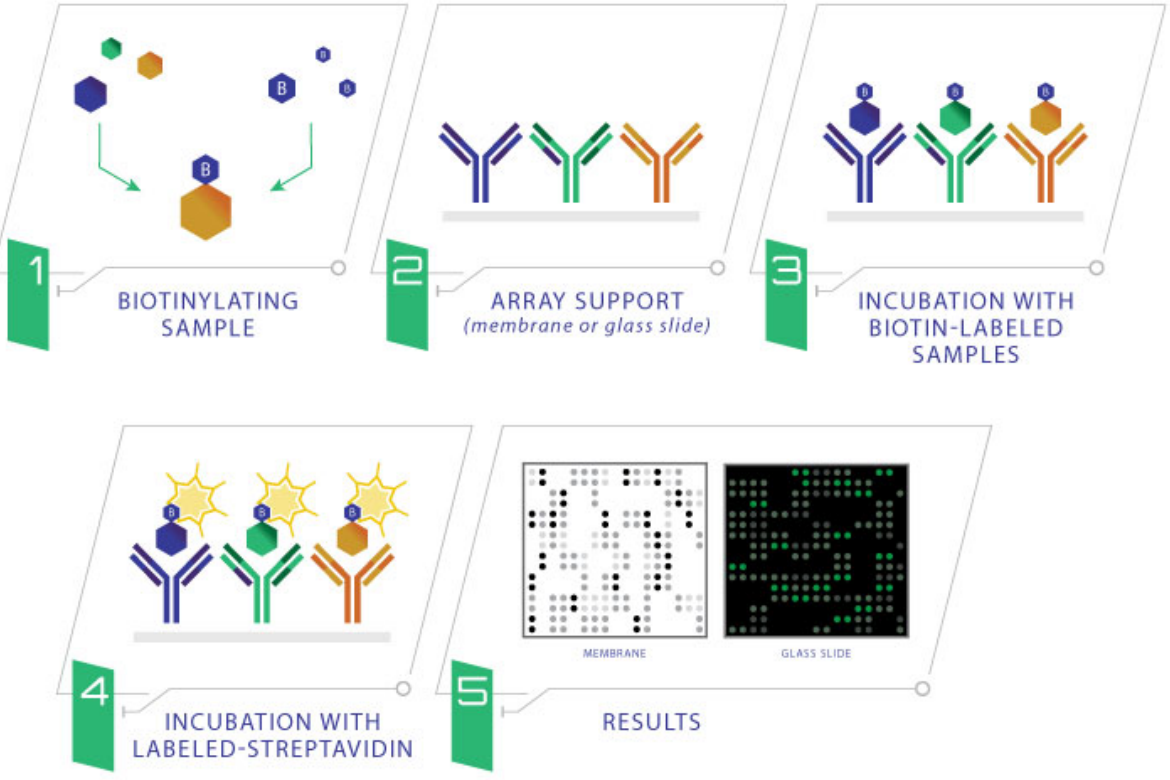
I. Introduction

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.

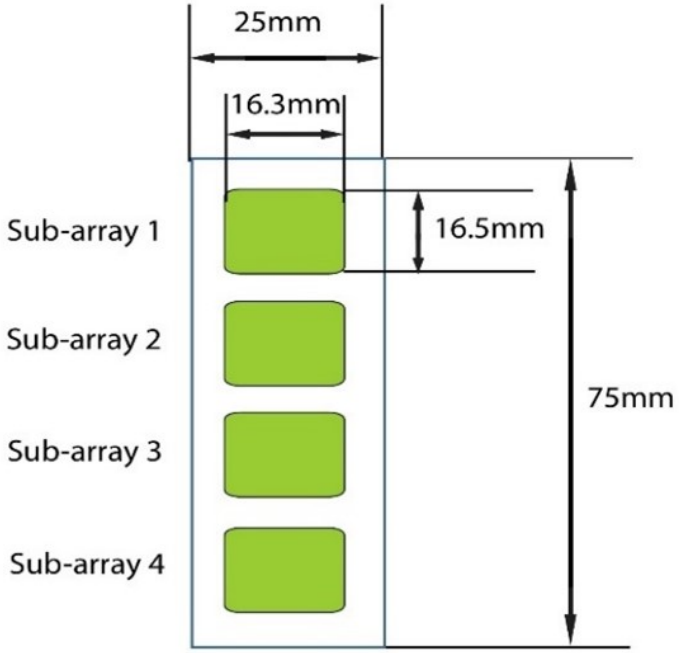
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

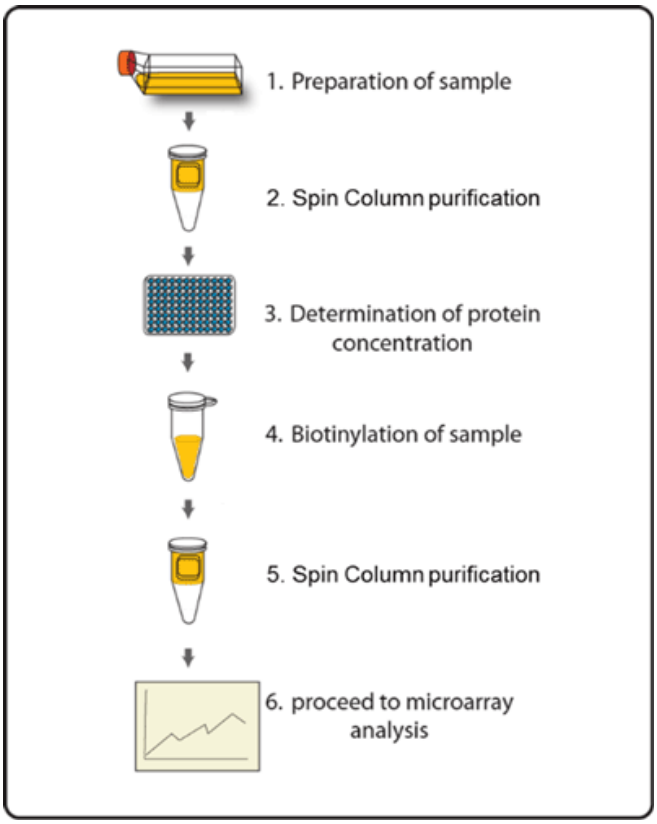
D. Incubations and Washes

- 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.

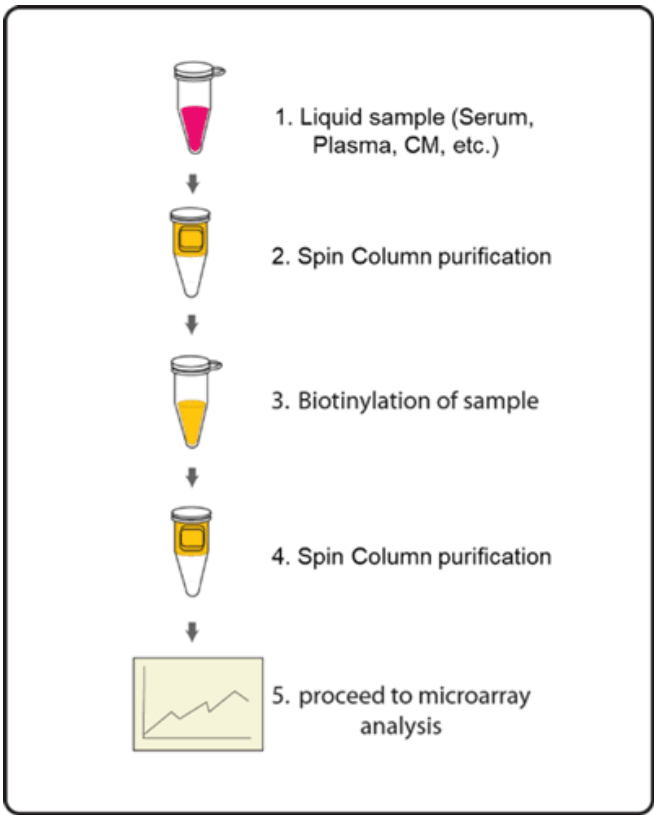
IV. Protocol

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.

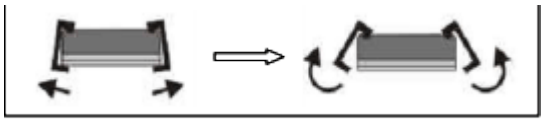
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

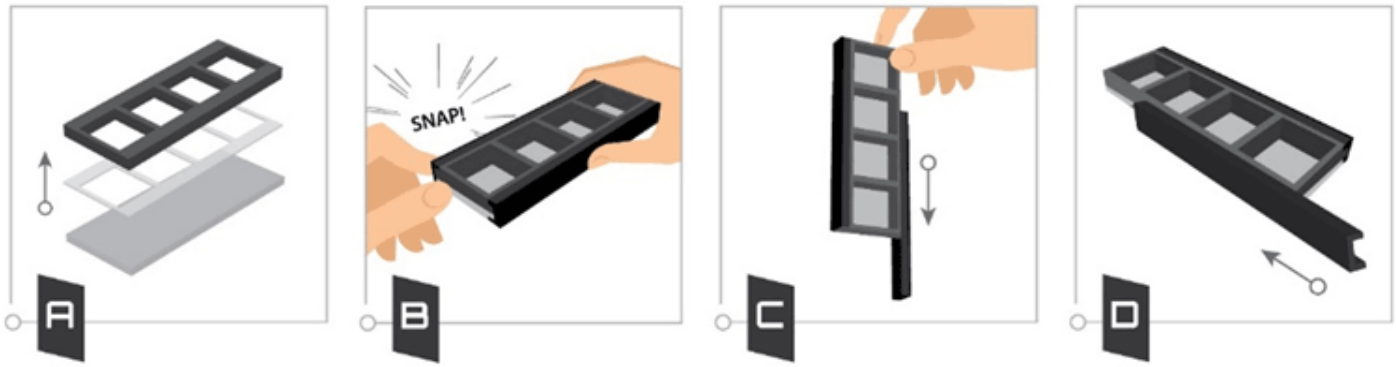
E. Fluorescence Detection

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	AARE	73	Filaggrin	145	PABP1	217	PREP	289	RPL22	361	SIM2	433	TRAP1
2	ACAT1	74	FITM1	146	PACS1	218	PRG2	290	RPL23A	362	SIRPB1	434	TRAP220
3	ACOT2	75	GARS	147	PNLIP	219	PrP	291	RPL3	363	Six3	435	TRF 2
4	ADAM28	76	GCC2	148	PARVB	220	Profilin 1	292	RPL32	364	SLC4A1	436	TRIM14
5	AHCY	77	GLI-2	149	PCAP	221	Prolargin	293	RPL4	365	SLITRK1	437	Tropomyosin 3
6	AK1	78	GLOD4	150	PCBP1	222	Prosaposin	294	RPL7	366	SLURP1	438	TRP-1
7	AKR1A1	79	GLUL	151	PCBP2	223	PTGDS	295	RPL7A	367	SMAD6	439	TRPS1
8	ALDH2	80	GMPR1	152	PCCA	224	PSMD2	296	RPLP0	368	SMC4	440	Trypsinogen-2
9	DEFA5	81	GOLGA3	153	PCDH12	225	Protein C	297	RPLP2	369	SMPD4	441	TSR2
10	ANKRD9	82	GP2	154	PCDH8	226	Protein Z	298	RPS10	370	SNRPD1	442	TTC3
11	ANXA3	83	gp340	155	PCK2	227	PRR4	299	RPS11	371	SOD1	443	TTF1
12	AP180	84	GTF2F1	156	PCMT1	228	PRRC2A	300	RPS12	372	SOD2	444	TUBA6
13	AP3S2	85	HA1	157	PCNA	229	PRSS23	301	RPS13	373	SOD-3	445	TWF2
14	APLP2	86	HARS	158	PCPE-1	230	PRSS3	302	RPS14	374	Somatoliberin	446	TXNDC15
15	ApoA V	87	HIC1	159	PCSK9	231	PRTN3	303	RPS15A	375	Somatostatin	447	TXNDC4
16	ASPM	88	HIP55	160	PDAP1	232	PSMA1	304	RPS16	376	SORD	448	TXNDC5
17	ASS1	89	H1F0	161	PDE1B	233	PSMA2	305	RPS18	377	SorLA	449	TXNRD2
18	ATOX1	90	HIST1H1B	162	PDIA6	234	PSMA4	306	RPS19	378	SOX4	450	UBA1
19	ATPG	91	HIVEP2	163	PDLIM1	235	PSMA5	307	RPS2	379	SOX5	451	UBE2D3
20	AUTS2	92	hnRNP K	164	PDLIM3	236	PSMA6	308	RPS20	380	SP-D	452	Ube2L3
21	BAI2	93	hnRNP R	165	PDZD2	237	PSMB1	309	RPS23	381	Spectrin	453	UBE2N
22	BarX1	94	HNRNPUL2	166	PEBP1	238	PSMB2	310	RPS25	382	SPEN	454	UCH-L1
23	BBS1	95	HNRPA3	167	PEBP4	239	PSMB3	311	RPS3	383	SPG48	455	UFM 1
24	UBC9	96	HP1 gamma	168	PENK	240	PSMB4	312	RPS3A	384	SPINK5	456	UGGT
25	BLM	97	Importin 7	169	PEPD	241	PSMB5	313	RPS4X	385	SPS2L	457	CMPK1
26	BOLA2	98	Involucrin	170	perilipin-3	242	PSMB6	314	RPS5	386	SPTBN2	458	UNC13D
27	C10orf58	99	ISLR	171	Perilipin-1	243	PSMB7	315	RPS8	387	SPTLC1	459	UNC45A
28	CACNA1H	100	ITPR2	172	Periostin	244	PSMC3	316	RPS9	388	Src	460	UNC5H4
29	Calpain-2	101	ITPR3	173	Periplakin	245	PSMD1	317	RREB1	389	SSC5D	461	UPB1
30	CaMK2	102	KCNAB3	174	Peroxiredoxin-2	246	PSMD5	318	RSF1	390	STAT3	462	UQCRB
31	CaMK2D	103	LAMA5	175	Peroxiredoxin-3	247	PSMD9	319	RSU1	391	Stathmin 1	463	UQCRH
32	CBL	104	LDB3	176	Peroxiredoxin-1	248	PSME1	320	RUSC1	392	STI1	464	URB
33	CBR1	105	LHPP	177	PFAS	249	PSME2	321	Septin 7	393	STOM	465	URB2
34	CCDC58	106	LIPG	178	PFDN6	250	PTBP1	322	S100A1	394	STXBP2	466	UROC1
35	CCT6A	107	MAP4K4	179	PFKL	251	PTEN	323	S100A11	395	SUCLG1	467	UROD
36	CHCHD3	108	MICALL2	180	PGAM1	252	PTGR1	324	S100A7	396	SUMO3	468	Uroguanylin
37	Cingulin	109	MON2	181	PGAM2	253	PTK7	325	S100A9	397	SVEP1	469	URP2
38	CIT	110	MPST	182	PGK-1	254	PTMA	326	SDC4	398	Symplekin	470	USP14
39	CMG1	111	MRC2	183	PGLS	255	PTPRG	327	SAA4	399	SynCAM	471	USP2
40	CNBP	112	MSH3	184	PG-M	256	PTPRK	328	SBP-1	400	Synemin	472	USP5
41	CNPY2	113	MTA2	185	PGM1	257	PTPRM	329	SC35	401	SYNPO2L	473	Uteroglobin
42	Coilin	114	MTHFD1	186	PGRPL	258	PTPRZ	330	SCG	402	Syntaxin 7	474	Utrophin
43	COL8A2	115	MUC5B	187	PHGDH	259	PZP	331	SCN3A	403	TAB182	475	VARS
44	COLEC11	116	MVD	188	Piccolo	260	QARS	332	SCP2	404	Talin1	476	VAP-1
45	COPG2	117	Myosin IIB	189	pIgR	261	QDPR	333	SDNSF	405	TARS	477	VAP-A
46	CORO1B	118	NACA1	190	PIK3C2B	262	QPRT	334	SDPR	406	TAX1BP3	478	VCP
47	CPA3	119	NAGPA	191	PIN	263	Quiescin Q6	335	SECISBP2	407	TBCA	479	VDAC1
48	CPI17 alpha	120	NAV2	192	PIP5K2 alpha	264	Rab1A	336	Secretogranin V	408	TCEB2	480	VILIP3
49	CrkRS	121	NFATC4	193	PISD	265	Rab7a	337	Semaphorin 6B	409	Tcf20	481	Vimentin
50	CRLF3	122	NNT	194	PLA2G1B	266	Ran	338	Semaphorin 7A	410	TCP1 delta	482	VNN1
51	CSRP3	123	NPEPPS	195	Plastin 3	267	RanBP1	339	SERBP1	411	TCP1 eta	483	VPS4B
52	CTNNAL1	124	NQO2	196	Plastin L	268	RanGAP1	340	Serpin A11	412	TCP1 theta	484	VSIG4
53	CTNND1	125	NSFL1C	197	PLBD2	269	RAP1B	341	Serpin A7	413	TCTP	485	WDR1
54	Cyclophilin F	126	NUCB1	198	PLD4	270	Rbm15	342	Serpin B3D	414	TDIF2	486	WDR44
55	Cystatin C	127	NUP214	199	Plectin	271	RCL	343	Serpin B6	415	Tenascin C	487	WISP2
56	DCAMKL1	128	OAF	200	Plexin B1	272	RECQ4	344	Serpin B8	416	Tenascin XB	488	WNK2
57	Dematin	129	OIT3	201	Plexin B2	273	Reg3A	345	Serpin F2	417	TFF2	489	XPG
58	DIAPH1	130	OPCML	202	PLOD1	274	REV3L	346	Serpin H1	418	TGM3	490	YB1
59	DKC1	131	ORM2	203	PLOD2	275	RHOC	347	Serpin A10	419	Thioredoxin-1	491	SYN1
60	DLST	132	OSBP1	204	Plxdc2	276	RHOG	348	SERPINB1	420	THOP1	492	YY1
61	DMRT1	133	OSCAR	205	PMCA	277	RNASE1	349	SerpinB4	421	TIF1 alpha	493	ZAK
62	Dystrophin	134	OSM R beta	206	PNP	278	RNASET2A	350	SerpinE2	422	TMEM103	494	zbtb11
63	Ebf4	135	Osteoadherin	207	POLD2	279	RLF	351	SerRS	423	TOB2	495	ZBTB4
64	EBP50	136	OTC	208	POLR2A	280	RNASE4	352	SET	424	TOMM70A	496	ZC3H18
65	ECHDC1	137	OTUB1	209	POR	281	Rnose2	353	SEZ6L2	425	TOP2B	497	ZC3H4
66	EHHADH	138	OTUD7A	210	PPOX	282	RP1	354	SF20	426	TPD52L2	498	ZC3H8
67	EIF3D	139	OT-NPI	211	PPP1CC	283	RPL10	355	SHANK1	427	TPM4	499	ZNF295
68	eIF4A2	140	p16 ARC	212	PPP1R9A	284	RPL10A	356	SHC1	428	TPP1	500	Zyxin
69	eIF4GII	141	p23	213	PPP2R1B	285	RPL11	357	SHMT1	429	TPPP3		
70	ENDOD1	142	p39	214	PPP2R4	286	RPL12	358	SHOX	430	TPR		
71	EYA2	143	P4HB	215	PRCP	287	RPL14	359	SHP-1	431	TALDO1		
72	F8	144	p73	216	PRDM13	288	RPL17	360	Siglec-1	432	Transthyretin		

VII. Interpretation of Results:

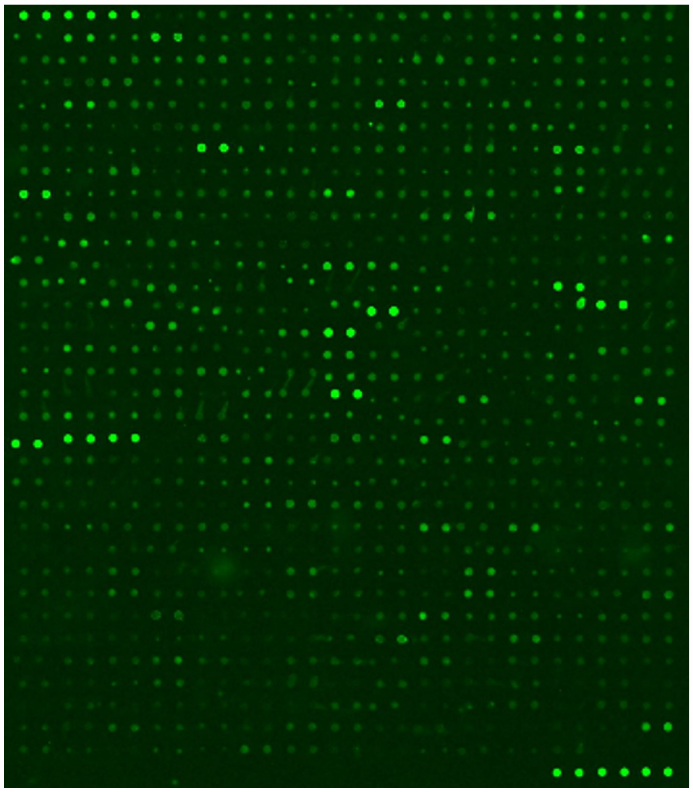
A. Explanation of Controls Spots

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.

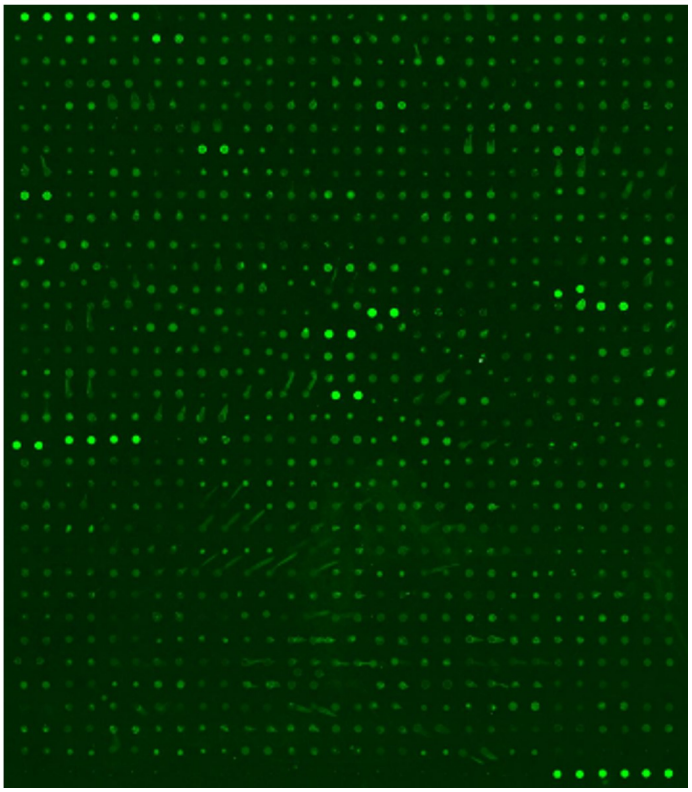
B. Typical Results

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

IX. Selected References

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