RayBio® Label-Based (L-Series) Rabbit L1 Array, Glass Slide

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAL-BLG-1-4 (4 Sample Kit)
AAL-BLG-1-8 (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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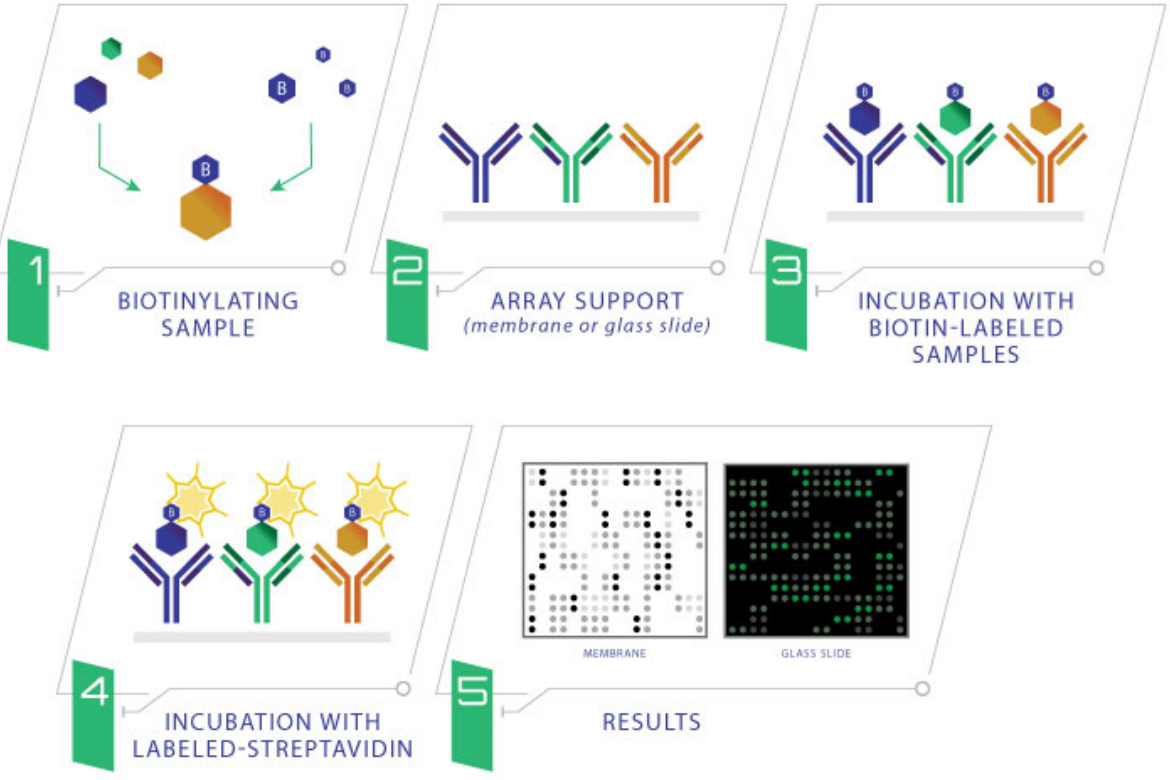
I. Introduction

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.

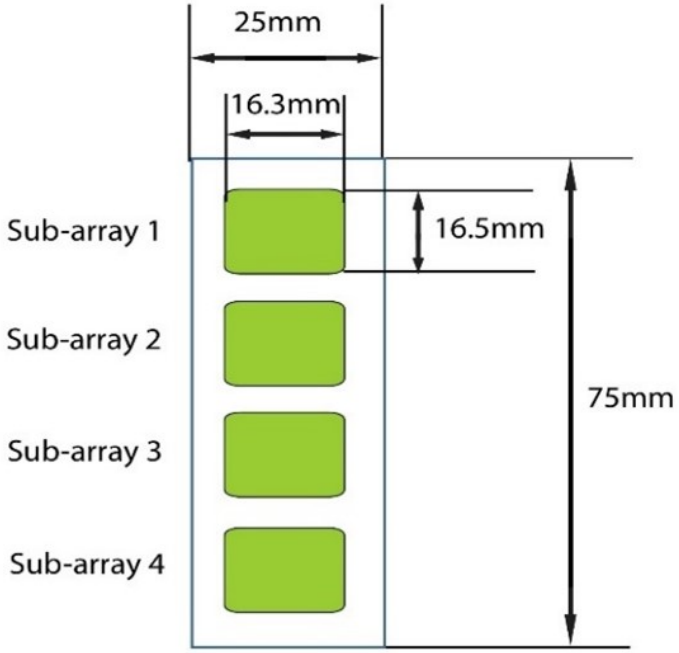
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

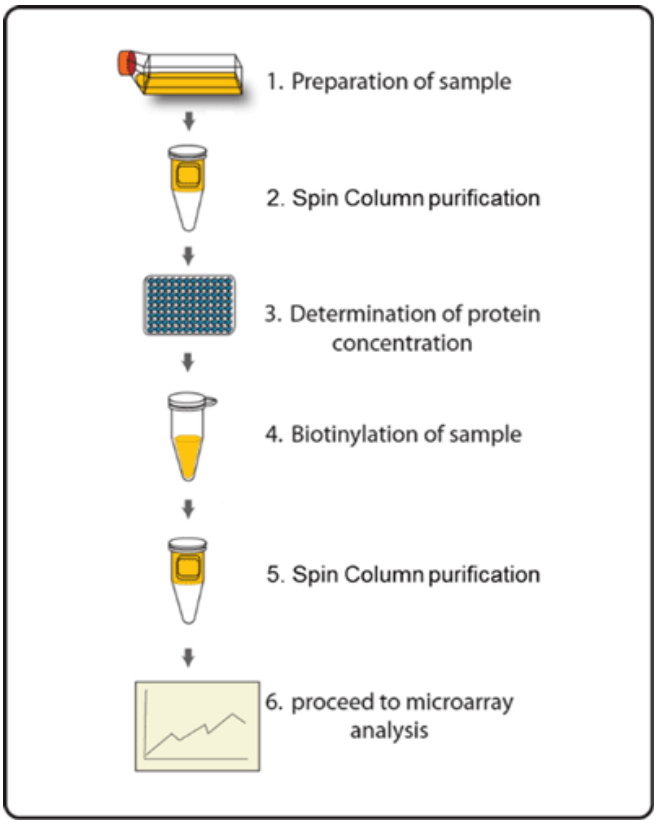
D. Incubations and Washes

- 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.

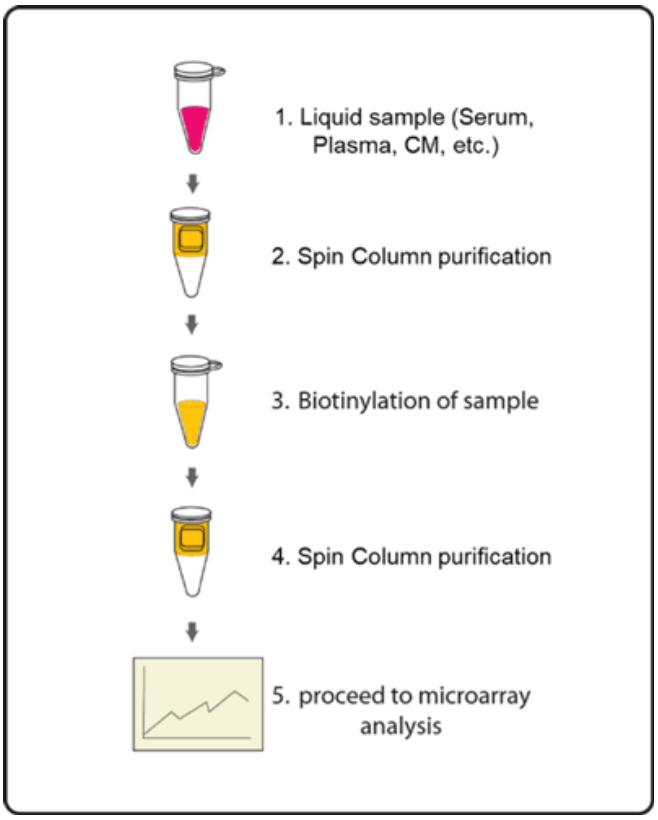
IV. Protocol

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.

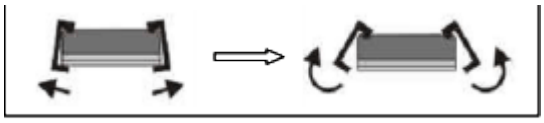
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

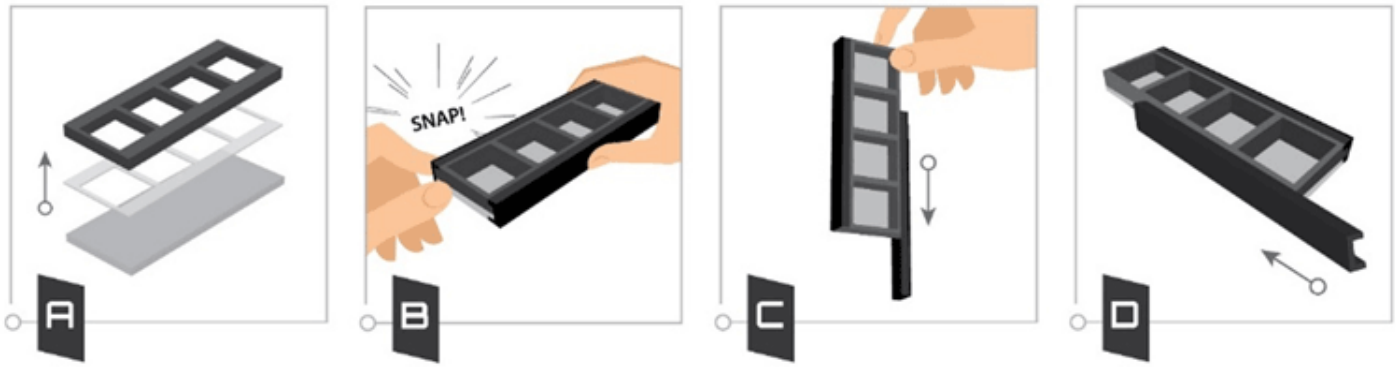
E. Fluorescence Detection

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
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	11b-HSD1	73	CD30	145	EVC2	217	Histone H1.3	289	LECT2	361	PDX-1	433	SPARC
2	4-1BB	74	CD36	146	EXTL2	218	Histone H3.3	290	LEDGF	362	PEBP1	434	SP-D
3	6Ckine	75	CD38	147	FABP4	219	HMGB1	291	Leptin	363	Pentraxin-3	435	Src
4	ACE-2	76	CD90	148	Factor IX	220	hnRNPL	292	Leptin R	364	Periostin	436	SREC-II
5	ACTC1	77	CD97	149	Factor VII	221	hnRNPU	293	LIF	365	PF4	437	SSEA-1
6	Activin R1B	78	CD98	150	Factor XI	222	HOXB3	294	LIF R alpha	366	PFAS	438	SSTR2
7	Activin R2A	79	CDSN	151	Factor XII	223	HOXD11	295	Lipocalin-2	367	PGM1	439	SVEP1
8	ADAMTS1	80	Cerberus 1	152	FAM3C	224	HPRT	296	LIX	368	PGRP-S	440	TCP1 eta
9	ADAMTS15	81	CFHR5	153	Fas Ligand	225	HRG	297	LRP-6	369	PHGDH	441	TFF1
10	ADAMTS4	82	Chemerin	154	FASN	226	HRG-beta 1	298	LSAMP	370	PLA2G1B	442	TGF beta 3
11	ADAMTS5	83	Chitotriosidase	155	FGF Basic	227	HSP10	299	L-Selectin	371	Plasminogen	443	TGF beta R2
12	Adiponectin	84	Chymase	156	FGF R4	228	HSP90	300	LTB	372	PLOD2	444	TGF beta R3
13	Aggrecan	85	CKB	157	FGF-10	229	HTRA1	301	LTBR	373	PNP	445	TGF-alpha
14	AHCY	86	CKMB	158	FGF-11	230	HTRA2	302	LYVE-1	374	Podocalyxin	446	TGF-beta 2
15	AK1	87	Claudin-4	159	FGF-16	231	ICAM-1	303	Matrilin-3	375	POR	447	TGF-beta R1
16	ALDH1A1	88	CLC	160	FGF-19	232	ICAM-2	304	Mcl-1	376	PP	448	Thioredoxin-1
17	ALK	89	CMASP3	161	FGF-21	233	ICAM-5	305	M-CSF	377	PRCP	449	Thrombopoietin
18	Alpha 1 AG	90	COL19A1	162	FGF-23	234	IDH1	306	MDC	378	Pref-1	450	TIM-4
19	alpha-Synuclein	91	COL1A1	163	FGF-9	235	IFNAR1	307	Mer	379	PREP	451	TIMP-1
20	AMPKa1	92	Contactin-1	164	FGFR1 alpha	236	IFNg	308	Metavinculin	380	Presenilin 1	452	TIMP-2
21	AMSH	93	Contactin-4	165	FGFR2	237	IFNGR1	309	MFRP	381	Procalcitonin	453	TL1A
22	ANG-1	94	Cortactin	166	FGL2	238	IGF-2	310	MIP-1 beta	382	Prolactin	454	TLR1
23	ANG-2	95	CPA1	167	Fibronectin	239	IGFBP-1	311	MIP-3 beta	383	Prolargin	455	TLR2
24	Angiogenin	96	CRHBP	168	Fibulin 3	240	IGFBP-6	312	MMP-10	384	Prostasin	456	TLR3
25	ANGPTL2	97	CRTAC1	169	FKBP12	241	IL-12RB2	313	MMP-13	385	Protein C	457	TLR4
26	ANGPTL3	98	CRTAM	170	FKBP25	242	IL-13	314	MMP-16	386	PSMA1	458	TMEFF2
27	Annexin A5	99	CT-1	171	FKBP51	243	IL-16	315	MMP-2	387	PTEN	459	TNFa
28	Annexin A7	100	CTLA-4	172	FLRG	244	IL-17A	316	MMP-7	388	PTH	460	TOP2B
29	ApoA1	101	CXCL3	173	Follistatin	245	IL-17F	317	MMP-8	389	PTP gamma	461	TPP1
30	ApoA2	102	CXCL9	174	Frizzled-4	246	IL-17RA	318	MMP-9	390	PTP mu	462	TRADD
31	ApoB	103	CXCR1	175	Frizzled-6	247	IL-17RB	319	MP1	391	PTPRZ	463	TRANCE
32	Artemin	104	CXCR2	176	FSH	248	IL-17RD	320	MPO	392	RANTES	464	TRAP220
33	B3GNT1	105	CXCR3	177	FSTL1	249	IL-19	321	MRP 1	393	Ras	465	TROY
34	Bax	106	CXCR4	178	FUCA1	250	IL-1a	322	MSH6	394	RBP4	466	TSG-6
35	beta-I Tubulin	107	CXCR5	179	G6PD	251	IL-1b	323	MyBPC3	395	Reg1A	467	TSH
36	BLAME	108	CXCR6	180	Galectin-3	252	IL-1F6	324	Myoglobin	396	RELT	468	TSP-1
37	BLC	109	Cyclin D1	181	GALNT2	253	IL-1R1	325	NCAM-1	397	Resistin	469	TSP-2
38	BLMH	110	Cyclophilin B	182	GASP-1	254	IL-1R2	326	Nectin-3	398	ROBO4	470	TSP-4
39	BMP-1	111	Cystatin A	183	Gastrokine 1	255	IL-1R3	327	NET1	399	ROCK2	471	TWEAK
40	BMP-2	112	Cystatin B	184	GCSF	256	IL-1R4	328	Netrin-4	400	RREB1	472	TWEAK R
41	BMP-3	113	Cystatin C	185	GCSF R	257	IL-1R6	329	Neurokinin A	401	S100A1	473	TXNDC15
42	BMP-4	114	Cytochrome C	186	GDF5	258	IL-1RA	330	Neuropilin-1	402	S100A10	474	TXNDC5
43	BMP-6	115	DAN	187	Gephyrin	259	IL-2	331	Neuropilin-2	403	S100A11	475	Ubiquitin+1
44	BMP-9	116	DDAH1	188	GFR alpha-1	260	IL-20	332	Neurotrimin	404	S100A9	476	UCH-L1
45	BMPR-IB	117	D-Dimer	189	GFR alpha-2	261	IL-20RB	333	Nidogen-2	405	SCF	477	UNC5H4
46	BMPR-II	118	Decorin	190	GFR alpha-3	262	IL-21	334	Notch-1	406	SCF R	478	uPA
47	BRCA2	119	Desmocollin-2	191	GHR	263	IL-22	335	Notch-3	407	SCGF	479	uPAR
48	C1QA	120	Desmocollin-3	192	GLI-2	264	IL-22BP	336	NPTXR	408	Serpin A12	480	UROC1
49	C1QC	121	Desmoglein-2	193	GLIPR2	265	IL-23	337	NR3C3	409	Serpin A3	481	UROD
50	C3a	122	DISC 1	194	GLO-1	266	IL-23R	338	NrCAM	410	Serpin B4	482	USP14
51	C5a	123	DKK-1	195	Glut2	267	IL-26	339	OBCAM	411	Serpin B5	483	USP2
52	CA1	124	Dystroglycan	196	Glut3	268	IL-27RA	340	Olfactomedin-1	412	Serpin B8	484	Uteroglobin
53	CA3	125	E-Cadherin	197	Glut5	269	IL-4	341	OSCAR	413	Serpin C1	485	VAP-1
54	CA9	126	EDA-A2	198	Glycoprotein V	270	IL-6	342	OSM R beta	414	Serpin D1	486	VARS
55	Cadherin-6	127	EDG-1	199	Glypican 3	271	IL-7	343	Osteoactivin	415	Serpin F1	487	VE-Cadherin
56	CALD1	128	EG-VEGF	200	GM-CSF	272	IL-7RA	344	Osteoadherin	416	Serpin F2	488	VEGF-A
57	CapG	129	Endocan	201	GMF beta	273	IL-8	345	Osteopontin	417	SerRS	489	VEGF-C
58	Caspase-14	130	Endoglin	202	gp340	274	IL-9R	346	Osteoprotegerin	418	sFRP-4	490	VEGFR1
59	Catalase	131	Endorepellin	203	GPD1	275	ILK	347	OX40 Ligand	419	SHP-1	491	VEGFR2
60	Cathepsin A	132	EN-RAGE	204	GPLD1	276	INSRR	348	P20Sb3	420	Siglec-1	492	VEGFR3
61	Cathepsin B	133	Eotaxin-1	205	GPR-39	277	Insulysin	349	p27	421	SLITRK1	493	Versican
62	Cathepsin G	134	EpCAM	206	Grb2	278	Integrin beta 6	350	P4HB	422	Smad 1	494	VILIP3
63	CCL3	135	EPCR	207	GRP75	279	IP-10	351	p53	423	Smad 4	495	Vimentin B
64	CCR1	136	EphA1	208	GRP78	280	I-TAC	352	PAK7	424	Smad 5	496	Vitronectin
65	CCR6	137	EphA2	209	H6PD	281	Kallikrein 5	353	PCNA	425	Smad 7	497	VSIG4
66	CCR7	138	EphB6	210	HABP2	282	Kallikrein 7	354	PCPE-1	426	SMPD4	498	WISP-1
67	CCR8	139	Ephrin-A1	211	HAI-1	283	Kallikrein 8	355	PCSK9	427	SOD2	499	XIAP
68	CCR9	140	Epigen	212	Haptoglobin	284	KLKB1	356	PDAP1	428	SOD3	500	ZC3H4
69	CD109	141	Epiregulin	213	HBB	285	KPNB1	357	PDGF R beta	429	Somatostatin		
70	CD133	142	EPOR	214	HB-EGF	286	LAMB2	358	PDGF-BB	430	SorLA		
71	CD157	143	ErbB2	215	CGA	287	Layilin	359	PDGF-C	431	SOST		
72	CD23	144	ErbB4	216	HEXB	288	LBP	360	PD-L1	432	SOX2		

VII. Interpretation of Results:

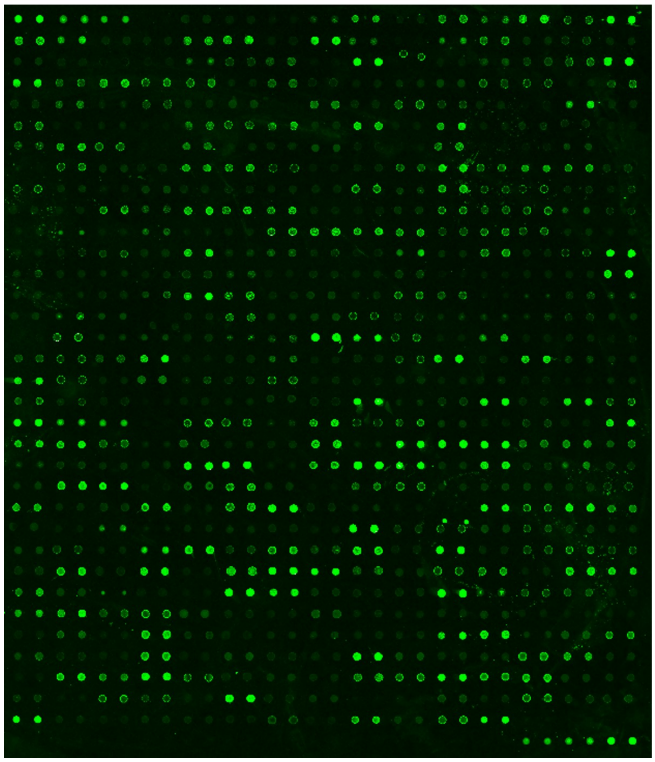
A. Explanation of Controls Spots

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.

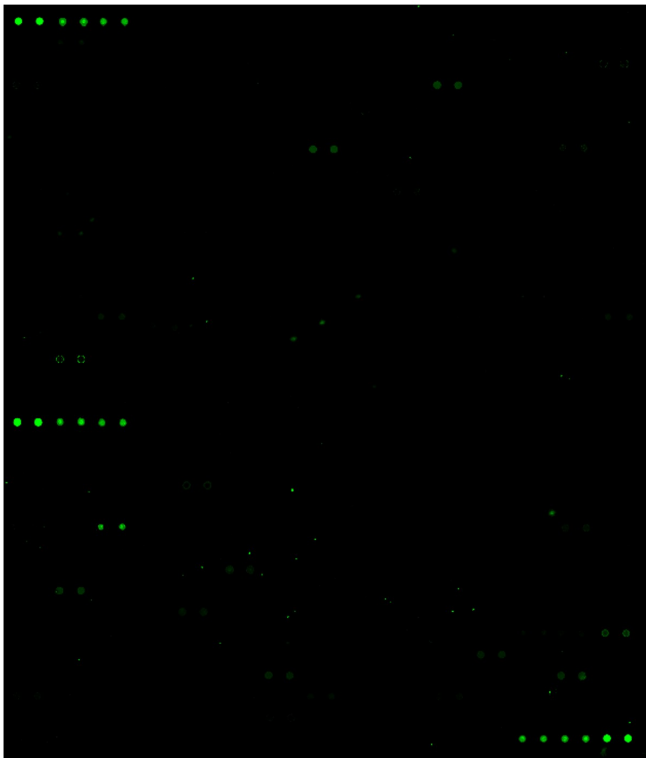
B. Typical Results

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.

Rabbit 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

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