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

Patent Pending Technology
User Manual (Jan 1, 2022)

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

AAR-BLG-3-4 (4 Sample Kit)
AAR-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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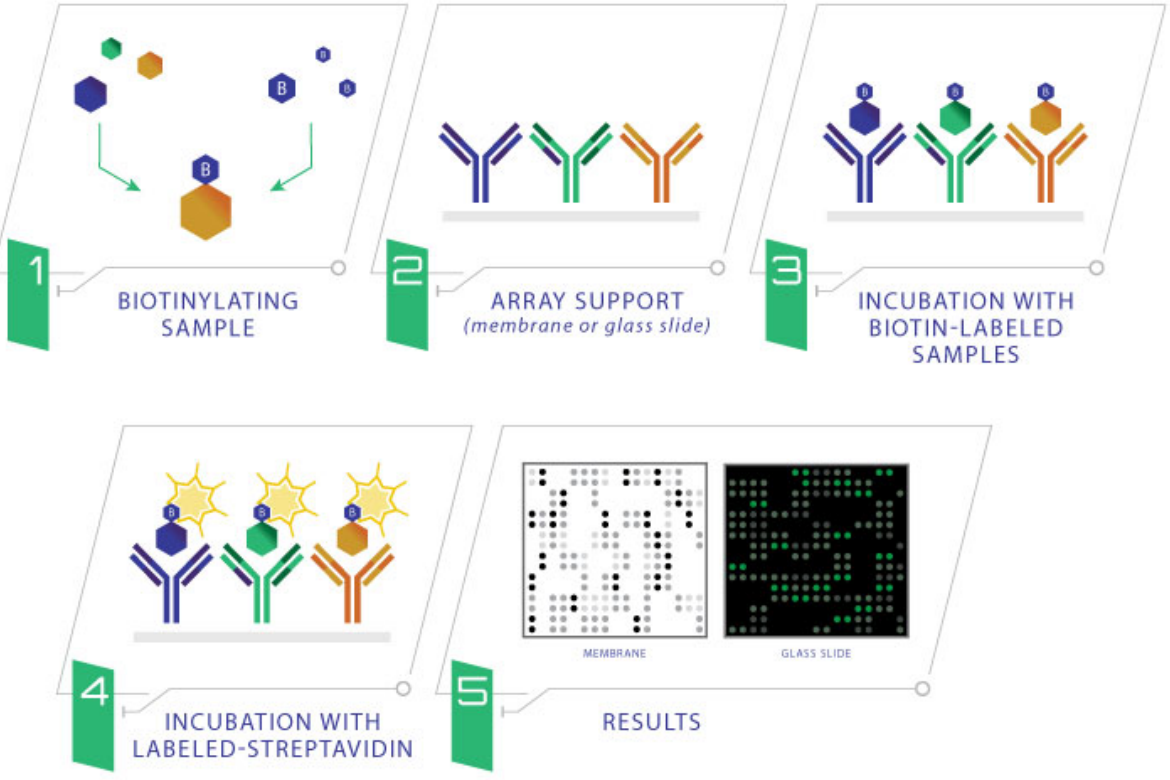
I. Introduction

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.

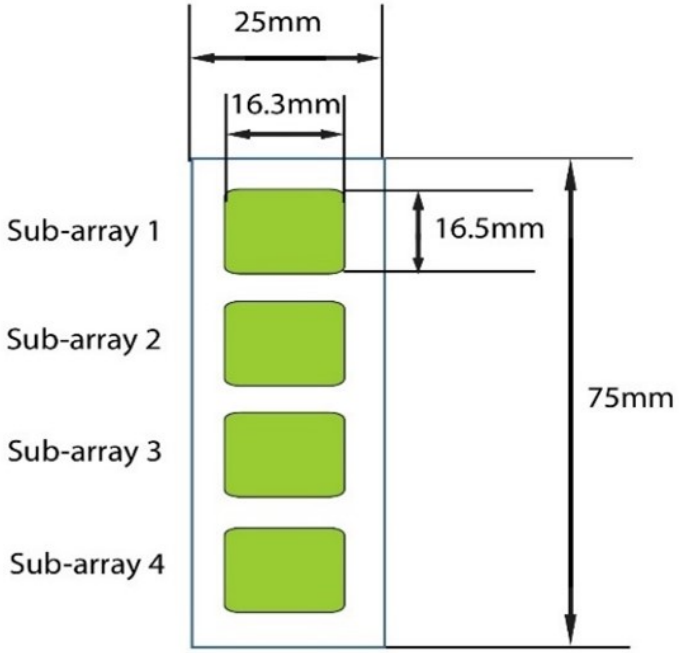
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

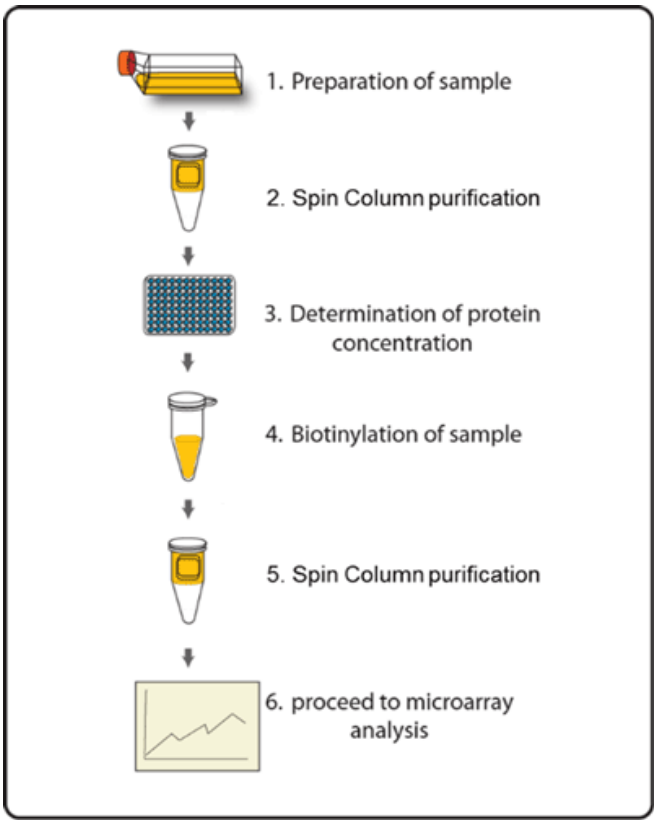
D. Incubations and Washes

- 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.

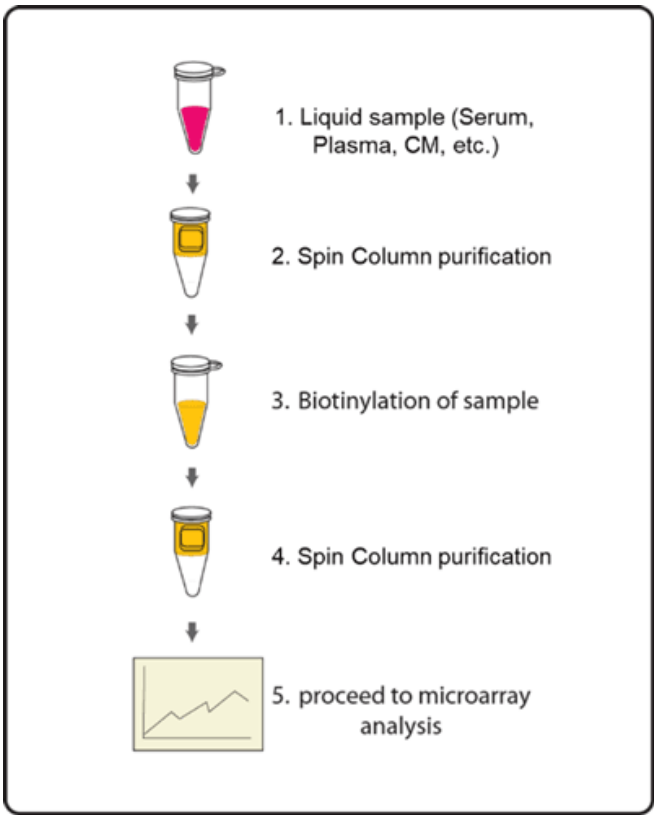
IV. Protocol

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.

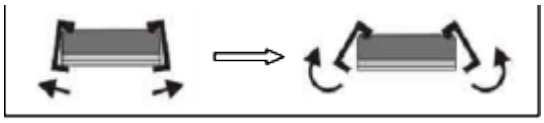
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

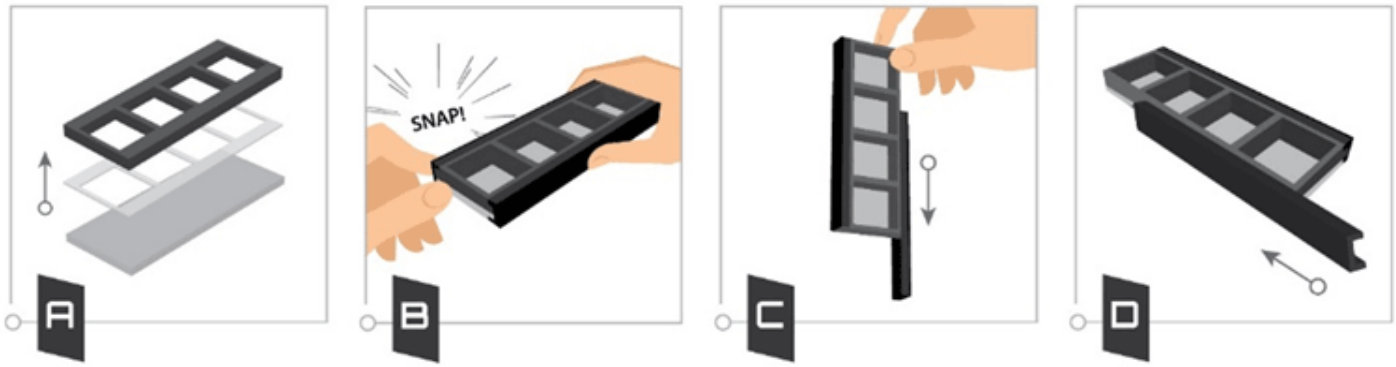
E. Fluorescence Detection

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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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	A2M	73	CHGB	145	FGFR5	217	IL-1 R4	289	Neurturin	361	Quiescin Q6	433	TCP1
2	AARE	74	Chk1	146	FGG	218	IL-1 R6	290	Nidogen-1	362	RAGE	434	TDIF2
3	ABCF1	75	Chymase	147	FH	219	IL-11	291	Nidogen-2	363	Ras	435	TECK
4	ACAT1	76	CINC-2	148	Fibronectin	220	IL-12 p40	292	NIT2	364	RELM beta	436	Tenascin X
5	Activin A	77	CINC-3	149	Ficolin-2	221	IL-12 RB1	293	NNT	365	Resistin	437	TFF1
6	ADAMTS10	78	Cingulin	150	FLG2	222	IL-13	294	NOV	366	REV3L	438	TFF2
7	ADAMTS15	79	CIP29	151	FOXN3	223	IL-15	295	NPB	367	Rheb	439	TGF-beta 1
8	ADAMTSL2	80	Claudin-3	152	Fractalkine	224	IL-16	296	NPTXR	368	RNASE6	440	TGF-beta 2
9	Aggrecan	81	Claudin-4	153	Frizzled-1	225	IL-17A	297	NR3C3	369	ROBO4	441	TGF-beta 3
10	AHCY	82	CNPY2	154	Frizzled-4	226	IL-17C	298	Nrf1	370	ROR1	442	TGF-beta R1
11	AHSG	83	CNTFR	155	Frizzled-5	227	IL-17D	299	OCT3/4	371	RP1	443	TGF-beta R2
12	Akt2	84	COL19A1	156	Frizzled-6	228	IL-19	300	Orexin A	372	RPL12	444	TIMP-1
13	Albumin	85	COTL1	157	Frizzled-7	229	IL-2 R beta	301	OSCAR	373	RPL23A	445	TIMP-2
14	AMPKa1	86	CPE	158	FSTL1	230	IL-24	302	OSM	374	RPLP0	446	Titin
15	ANGPTL2	87	CRADD	159	Galanin	231	IL-27	303	Osteoactivin	375	RPS13	447	TK1
16	ANGPTL3	88	CREB	160	GASP-1	232	IL-28B	304	Osteoadherin	376	RPS14	448	TLR1
17	ANKRD9	89	CRF21	161	GASP-2	233	IL-3	305	Osteoprotegerin	377	RPS15A	449	TLR3
18	ANXA6	90	CRHBP	162	G-CSF R	234	IL-3 R beta	306	p130Cas	378	RPS23	450	TLR4
19	APBA2	91	CrkRS	163	GDF-15	235	IL-5	307	p21	379	RPS3A	451	TMEFF1
20	ApoA1	92	CRTAC1	164	GDF-5	236	IMP2	308	P4HB	380	RPS5	452	TMEFF2
21	ApoA2	93	CRTAM	165	GFRA4	237	INSL3	309	Pappalysin-1	381	RPS8	453	TMEM223
22	ApoB	94	CRTH-2	166	GHR	238	Inuslin	310	PCAP	382	RPS9	454	TOMM70A
23	ApoE	95	Cryptic	167	GKN1	239	I-TAC	311	PCPE-1	383	RREB1	455	TPIS
24	ARHGAP1	96	CSE1L	168	GLI-2	240	Jak2	312	PD-1	384	RSF1	456	TPP1
25	ATG5	97	CSK	169	GLIPR2	241	Kallikrein 10	313	PD-ECGF	385	RUSC1	457	TRADD
26	ATPG	98	CTNND1	170	Glut1	242	Kallikrein 11	314	PDGF-AA	386	S100A10	458	TRAIL R2
27	B3GAT1	99	CXCR2	171	Glut2	243	Kallikrein 5	315	PDGF-C	387	S100A11	459	TRAM
28	B4GalT1	100	CXCR4	172	Glut4	244	Kallikrein 6	316	PDGF-D	388	S100A9	460	TRIM14
29	B7-1	101	CXCR7	173	Glut5	245	KIF5B	317	PDGFRB	389	S-100b	461	Tropomyosin 3
30	B7-H2	102	Cyclin D1	174	GM2A	246	LAMA5	318	PDLIM5	390	SBP-1	462	TRRAP
31	BAFF R	103	Cyclophilin F	175	GM-CSF	247	LAMP	319	PDZD2	391	SCF	463	Trypsinogen-2
32	Bax	104	Cystatin A	176	GP2	248	LASP1	320	PENK	392	SCF R	464	TSLP
33	BDNF	105	Cystatin B	177	gp340	249	LBP	321	Pentraxin-3	393	SDF4	465	TSP-1
34	beta-NGF	106	Cystatin D	178	GPD1	250	Lefty-1	322	Perilipin-3	394	Septin-7	466	TSP-2
35	BLAME	107	Cystatin E	179	GPR-39	251	Lefty-A	323	Peroxiredoxin-3	395	SERBP1	467	TSP-4
36	BLMH	108	Cystatin S	180	Granzyme A	252	LHPP	324	PF4	396	Serpin A3	468	TTF1
37	BMP-1	109	DAK	181	Granzyme M	253	LIX	325	PFAS	397	Serpin A5	469	TUBA6
38	BMP-15	110	DCI	182	GRHPR	254	LPS	326	PFDN6	398	Serpin B5	470	TWF2
39	BMP-9	111	DCXR	183	GRP	255	LRG1	327	PHGDH	399	Serpin C1	471	TXNDC15
40	BNIP2	112	DLL4	184	GSK-3 beta	256	LRP-6	328	Piccolo	400	SET	472	TXNDC5
41	BOLA2	113	DMGDH	185	GSN	257	L-Selectin	329	PIK3R2	401	sFRP-4	473	TYRO10
42	BTC	114	DSCAM	186	GSR	258	LUZP1	330	PINCH1	402	SH3BGRL3	474	UBC9
43	BTF3	115	DSG1	187	GSTM1	259	Lymphotactin	331	PIP4K2A	403	SHBG	475	Ubiquitin
44	C1q	116	EDA-A2	188	GSTO1	260	MAdCAM-1	332	PLA2G1B	404	SHOX	476	Ubiquitin+1
45	C1s	117	EDAR	189	GULP1	261	MAN1	333	PLD4	405	Siglec-1	477	UNC45A
46	C3a	118	eEF2	190	HAI-1	262	Mcl-1	334	Plexin B2	406	SLC38A10	478	UNC5H4
47	C5a	119	EG-VEGF	191	Haptoglobin	263	MCP-1	335	PlGF-2	407	SLITRK1	479	uPA
48	CA1	120	eIF4E	192	HB-EGF	264	MCP-5	336	PLS3	408	SLPI	480	UROC1
49	CA2	121	EMAP-II	193	HEG1	265	MDC	337	PNP	409	SLURP1	481	USP2
50	CA3	122	Endothelin	194	Hepassocin	266	MEP1A	338	POMC	410	Smad 1	482	Uteroglobin
51	Calbindin D	123	Eotaxin-2	195	HEXB	267	Mesothelin	339	PON1	411	Smad 4	483	VAP-1
52	Cardiotrophin-1	124	EphA1	196	HGFA	268	MICB	340	PP	412	Smad 5	484	VAP-A
53	Cathepsin A	125	EphA2	197	Histone H2AY	269	MIP-3 alpha	341	PPP1CC	413	Smad 8	485	VARS
54	CCL28	126	EPHX2	198	hnRNPL	270	MIS RII	342	PRAT4B	414	Somatostatin	486	VDAC1
55	CCR3	127	Epiregulin	199	Hoxb3	271	Mitofusin 2	343	PRELP	415	SOX5	487	VEGF
56	CCR4	128	ERRa	200	HOXD11	272	MKK3	344	Prolactin R	416	SPARC	488	VEGF-B
57	CCT3	129	E-Selectin	201	HSP10	273	MKK4	345	ProSAAS	417	SPINK7	489	VEGF-C
58	CD133	130	EVC2	202	HSP47	274	MMP-10	346	Prostasin	418	SPTBN5	490	VEGFR3
59	CD23	131	Factor IX	203	HTRA1	275	MMP-13	347	Protein Z	419	SSTR2	491	VILIP3
60	CD24	132	Factor V	204	HVEM	276	MMP-16	348	Prouroguanylin	420	STXBP2	492	Visfatin
61	CD2AP	133	Factor VII	205	ICAM-1	277	MMP-7	349	PRR4	421	SVEP1	493	Vitronectin
62	CD30	134	Factor XII	206	ICAM-2	278	MRP 1	350	PRRC2A	422	SYK	494	WARS
63	CD40 Ligand	135	FAM3C	207	IDE	279	Multimerin 2	351	PRTN3	423	SYN1	495	WISP-1
64	CD9	136	Fas	208	IFN-beta	280	MuSK	352	P-selectin	424	TACI	496	WISP-2
65	CD90	137	Fas Ligand	209	IFNGR1	281	MyBPC3	353	PSMB1	425	TAGLN2	497	XPD
66	CDC14	138	FGF-11	210	IGFBP-2	282	NACA1	354	PSMD2	426	TALDO	498	XPG
67	CFH	139	FGF-20	211	IGSF4C	283	NADK	355	PSMD9	427	TALDO1	499	YY1
68	CFI	140	FGF-23	212	IL-1 alpha	284	NAGPA	356	PSME1	428	Talin-2	500	ZC3H4
69	CFL1	141	FGF-9	213	IL-1 F10	285	NAPRT1	357	PTHLP	429	TARC		
70	CGA	142	FGF-BP	214	IL-1 F5	286	NeuroD1	358	PTMA	430	TARS		
71	CHCHD3	143	FGFR1	215	IL-1 F6	287	Neurolysin	359	PYY	431	TCA-3		
72	Chemerin	144	FGFR2	216	IL-1 F9	288	Neuropilin-2	360	QARS	432	Tcf20		

VII. Interpretation of Results:

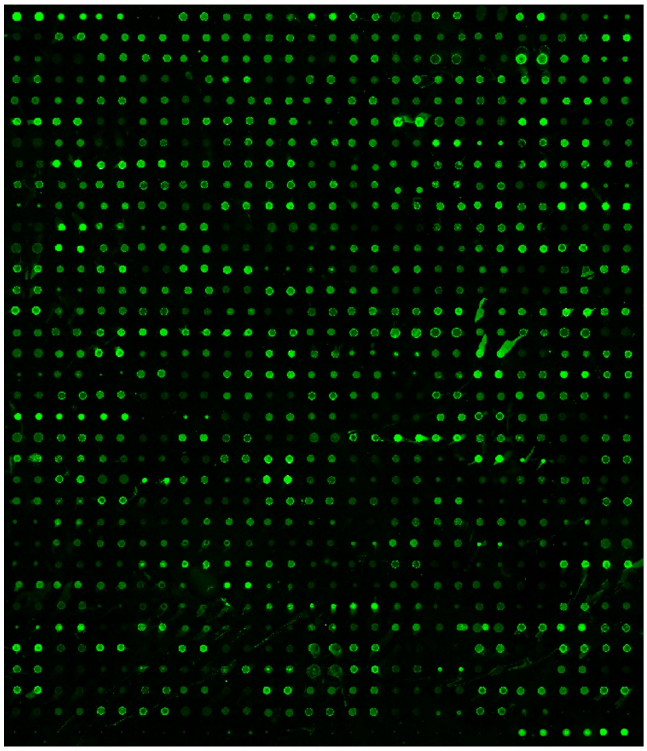
A. Explanation of Controls Spots

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.

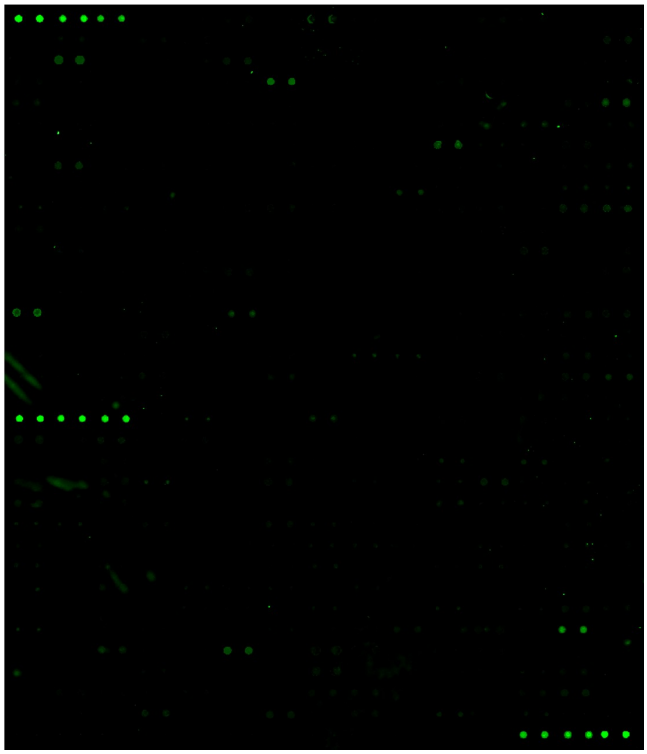
B. Typical Results

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.

Rat Serum



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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