RayBio® Label-Based (L-Series) Rat L4 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-4-4 (4 Sample Kit)
AAR-BLG-4-8 (8 Sample Kit)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

Your Provider of Excellent Protein Array Systems and Services

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Tel: +1-770-729-2992 or 1-888-494-8555 (Toll Free); Fax: +1-770-206-2393;
Website: www.raybiotech.com Email: info@raybiotech.com

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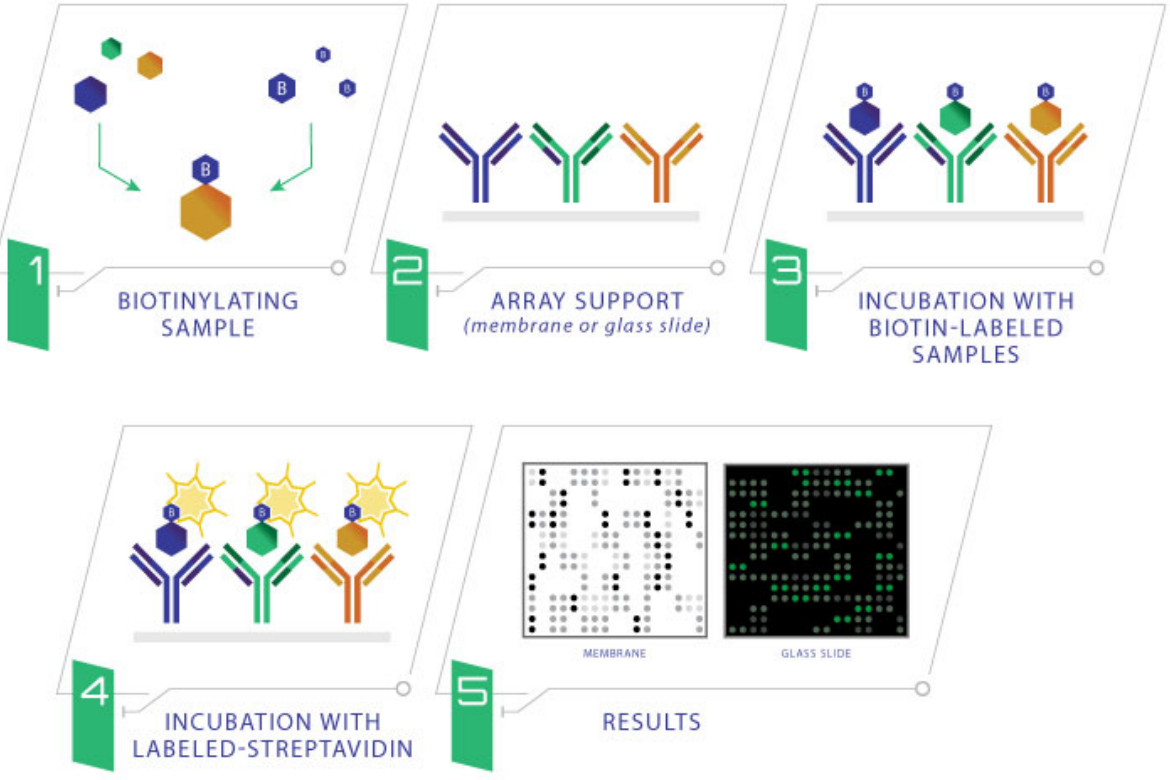
I. Introduction

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.

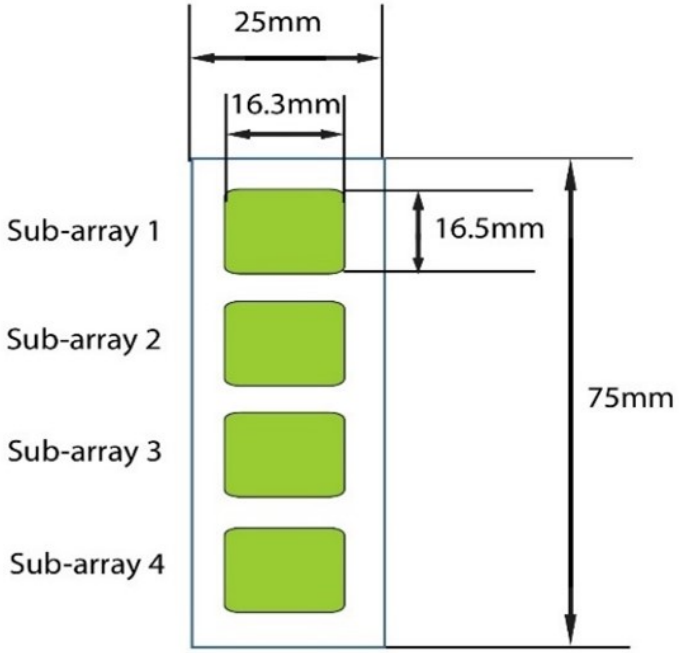
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

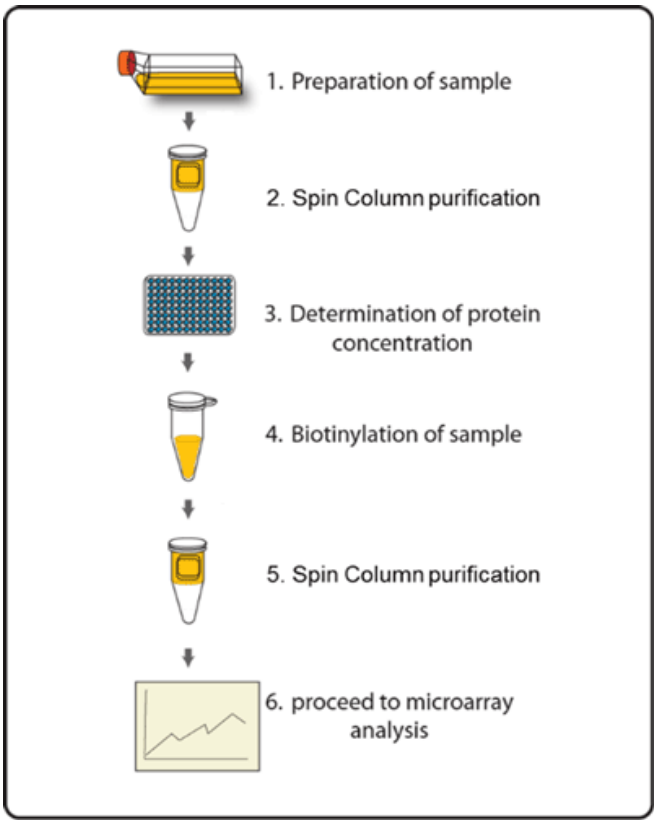
D. Incubations and Washes

- 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.

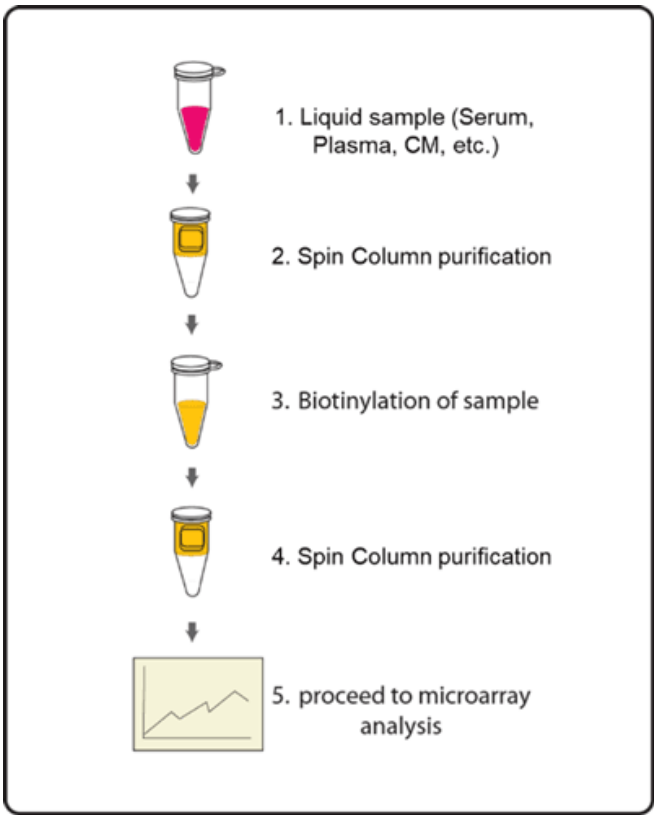
IV. Protocol

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.

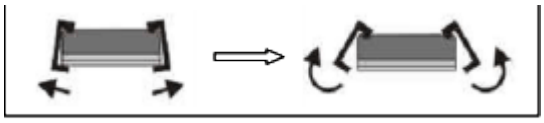
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

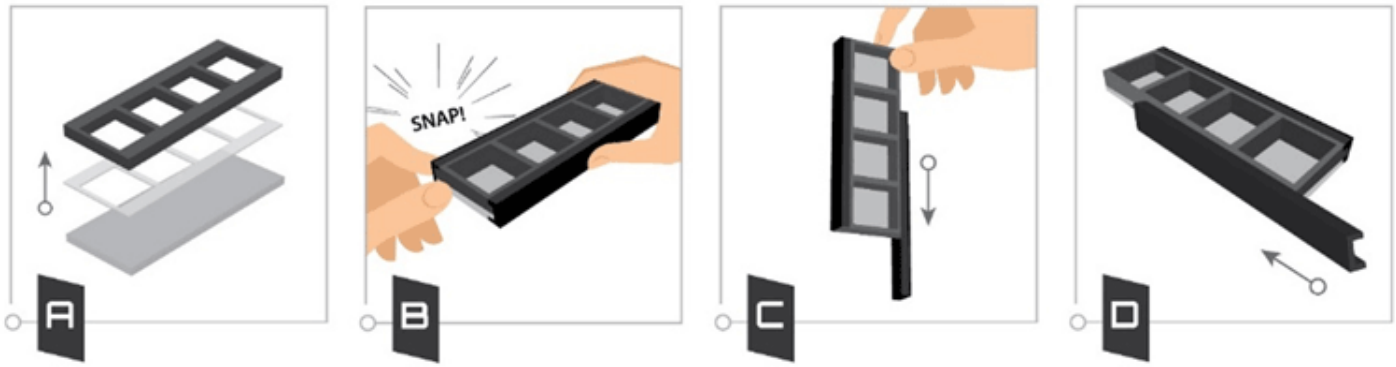
E. Fluorescence Detection

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
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VI. Antibody Array Target List

Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	14-3-3 beta	73	CD40	145	FCGR3A	217	IMPAD1	289	NDFIP1	361	PNUTS	433	SHIP
2	14-3-3 gamma	74	CD44	146	FCGRT	218	IMPDH2	290	Nectin-3	362	PP2A	434	SHP-2
3	A1BG	75	CD51	147	Fen 1	219	Inhibin beta	291	Nesfatin-1	363	PPM1B	435	SIGNR3
4	A1M	76	CD59	148	Filamin A	220	iNOS	292	Nesprin2	364	PPM1L	436	Six3
5	aAmylase	77	CDC25A	149	FKBP38	221	Intelectin-1	293	Neurogenin-2	365	PPP1R9B	437	SMAGP
6	ACE2	78	CDC25C	150	FoxA2	222	IRE1	294	Neuroglycan C	366	PRCP	438	SMOC-1
7	ACLP	79	CDK1	151	FoxP3	223	IRS1	295	NGFR	367	PRDX5	439	SMURF2
8	ACTN2	80	CDK2	152	FPRP	224	IRS2	296	Nicalin	368	PRG2	440	SNAP25
9	ADAM17	81	CEACAM1	153	FSTL4	225	ITGA8	297	Ninjurin-2	369	PRNP	441	SOD1
10	ADAM9	82	CELF1	154	FUCA1	226	ITGB4BP	298	NIPP1	370	Prohibitin	442	SOD2
11	ADNP	83	CES3	155	Fyn	227	ITGB5	299	NKX2.2	371	Prss21	443	SOD-3
12	ADRB2	84	CHORDC1	156	G3BP	228	ITGB6	300	NLRP10	372	PSD-95	444	SPOCK2
13	AFP	85	CKBB	157	G6PD	229	ITPR3	301	NPC1	373	PTEN	445	SQSTM1
14	AGT	86	CLEC1B	158	GABAB R1	230	JAB1	302	NR3C1	374	PTGDS	446	SR-AI
15	Akt1	87	CLEC5A	159	GABAB R2	231	Jak1	303	NrCAM	375	PTGES3	447	ST3GAL2
16	ALDH2	88	COL1A1	160	GABRA1	232	JIP1	304	NSE	376	PTP gamma	448	STAT5b
17	ALOX5	89	COL6A1	161	GABRA5	233	Kallikrein 7	305	NT5E	377	PTP-MEG2	449	STAT6
18	alpha 2u-Globulin	90	COLEC10	162	GALNT2	234	KCNB2	306	NUAK1	378	PTPRM	450	STI1
19	ALPP	91	Complexin-1	163	gamma Catenin	235	KCNC1	307	Nucleostemin	379	PTPRU	451	STIM1
20	AMBP	92	Contactin-3	164	GATE-16	236	KIAA1967	308	NXPH3	380	PVRL1	452	STK3
21	AMH	93	COPZ1	165	GBL	237	Klotho beta	309	Oligodendrocyte Marker O1	381	QDPR	453	Substance P
22	Amphiphysin	94	CPEB3	166	GDF1	238	KMO	310	Oligodendrocyte Marker O4	382	Rab11A	454	SUMO3
23	AMPK beta 1	95	CPM	167	GDF7	239	KOR	311	Oncomodulin	383	Rab27a	455	SUSD2
24	ANG-2	96	CSNK1A	168	GDI1	240	KPNB1	312	OPRM1	384	RAB7A	456	Synaptotagmin-1
25	Angiogenin	97	CSNK1D	169	Gephyrin	241	Kynureninase	313	Osteopontin	385	RAC1	457	Syndecan-3
26	Annexin A11	98	CSNK1E	170	GLUD1	242	LAMC1	314	OV-6	386	RACK1	458	Syntaxin 1B
27	Annexin A2	99	CSNK1G	171	Glycine R	243	Laminin S	315	P20Sb3	387	Rad17	459	Syntaxin 7
28	ApoH	100	CSNK2B	172	GOLGB1	244	LC3B	316	p38 alpha	388	Raf-1	460	Syntaxin 8
29	ARC	101	CSRP1	173	GPLD1	245	LHX5	317	p70 S6 Kinase	389	Rap1A/B	461	Syntaxin BP1
30	ATF2	102	CXCR3	174	GPR64	246	LIPG	318	PABP	390	Rap2A/B	462	T Cell Receptor alpha Ch
31	ATF6	103	CXCR6	175	GPX2	247	Lipin 2	319	PAK4	391	RECQ4	463	TCEB2
32	ATG3	104	CYTL1	176	GPX4	248	LMAN1L	320	PAK6	392	REG4	464	TCP1 eta
33	ATM	105	DARC	177	GRB7	249	LMNA	321	Pannexin-1	393	Relaxin R1	465	Tenascin R
34	Axin-1	106	DARPP-32	178	GRP78	250	LOK	322	Park7	394	RELM gamma	466	TfR
35	B7-H4	107	DDC	179	GSK-3 alpha	251	LRPAP	323	PARL	395	RGM-B	467	TGN38
36	BAG4	108	DDT	180	H6PD	252	Lumican	324	Parvalbumin	396	RGM-C	468	TH
37	BAG6	109	DDX1	181	HABP2	253	Lysozyme	325	Paxillin	397	RHOG	469	Themis
38	BAMBI	110	DEFA6	182	HAO-1	254	LYVE1	326	PCBP2	398	RIBP	470	Thioredoxin-1
39	BarX1	111	DGK-gamma	183	HBB	255	MAD2L1	327	PCDH12	399	RIPK1	471	Thrombopoietin
40	BCHE	112	DGK-theta	184	HCLS1	256	MafB	328	PCK2	400	RKIP	472	TLR7
41	Beclin 1	113	DISC 1	185	HDAC2	257	MAP4K4	329	PCNA	401	RNASE4	473	TOP2B
42	beta-Actin	114	Dkk-1	186	HDAC4	258	Matrilin-4	330	PCSK9	402	RNF2	474	TOR
43	beta-I Tubulin	115	Dkk-2	187	HHEX	259	MBP	331	PDAP1	403	ROCK1	475	TRIM63
44	BMX	116	DOCK1	188	HIBADH	260	MCHR1	332	PDCD5	404	RPL10A	476	Troponin T
45	BNIP3L	117	DOT1L	189	HIF-2 alpha	261	M-CSF	333	PDCD6	405	RPL11	477	TRP14
46	BOK	118	DRAK2	190	Histamine H3 R	262	MDGA2	334	PDHX	406	RPL22	478	TRPV1
47	Brevican	119	Draxin	191	Histone H1.3	263	MDH1	335	PDK-1	407	RPLP2	479	TRXR1
48	CA14	120	DSC2	192	Histone H2AX	264	MDM2	336	PDX-1	408	RPS11	480	Trypsin 3
49	Cadherin-15	121	DYRK1A	193	HMGB1	265	MEK1	337	PDZK1	409	RPS19	481	Trypsin Pan
50	Cadherin-8	122	Dystroglycan	194	HMGN2	266	MEKK2	338	Perilipin-1	410	RPS25	482	TSC22
51	CALD1	123	EDN	195	HMOX1	267	MESDC2	339	PGAM2	411	RPS4X	483	TSH
52	Calretinin	124	EFEMP2	196	HN1	268	Metallothionein	340	PGK1	412	RPS6	484	TXNDC4
53	CaM Kinase II	125	EGLN1	197	hnRNP G	269	mGluR1	341	PGLS	413	RRAS2	485	UBASH3B
54	CaMKK alpha	126	EIF3D	198	hnRNP U	270	mGluR2/3	342	PGM1	414	RSK1	486	UBE2N
55	CapG	127	ELAVL1	199	HOMER1	271	mGluR5	343	PGRP-S	415	RSK2	487	UQCRB
56	CART	128	Endoglin	200	HP1BP3	272	MIB1	344	PIK3R1	416	RTN1-A	488	UROD
57	Cathepsin G	129	Endophilin A1	201	HPRT	273	MIOS	345	PIWIL2	417	RYK	489	VAP-B
58	Caveolin-1	130	Endorepellin	202	HS6ST3	274	MIP-1 beta	346	PKA RI beta	418	SC35	490	VE-Cadherin
59	CBP	131	ENSA	203	HSP90B1	275	MKP-3	347	PKC beta 1	419	SCGB3A1	491	Versican
60	CCBL1	132	EpCAM	204	HSPA2	276	MLK4	348	PKC gamma	420	SCGF	492	Vimentin
61	CCR2	133	EphA3	205	HSPB8	277	MN1	349	PKLR	421	SEC13	493	WNK1
62	CCR6	134	Ephrin-B3	206	IBP160	278	MPP5	350	PKN2	422	SECISBP2	494	WNK2
63	CCR8	135	ERBB4	207	ICAM-5	279	M-Ras	351	PLA2G2A	423	SEMA3F	495	WT1
64	CCR9	136	ERK1	208	IKK alpha	280	MSH6	352	PLC-beta 4	424	SENP8	496	WWOX
65	CD106	137	ERK2	209	IKK gamma	281	Musashi-1	353	PLC-gamma 1	425	Serpin A12	497	XPB
66	CD161	138	ERK4	210	IL-12 R beta 2	282	MyD88	354	Plexin A1	426	Serpin A3N	498	YAP1
67	CD164	139	FAIM3	211	IL-17F	283	MYHC	355	Plexin A2	427	Serpin A6	499	Yes
68	CD19	140	FANCD2	212	IL17RA	284	Myoglobin	356	Plexin A3	428	Serpin D1	500	ZBTB4
69	CD28	141	Fascin	213	IL-20RB	285	NAP1L1	357	Plexin B3	429	SerpinE2		
70	CD29	142	FASN	214	IL-21R	286	Nbs1	358	PLOD2	430	SerRS		
71	CD36	143	FBPase 1	215	IL-6R	287	NCAM2	359	PLTP	431	SGSH		
72	CD39L4	144	FCGR2B	216	IMPA1	288	NCOR1	360	PNPLA2	432	SHC1		

VII. Interpretation of Results:

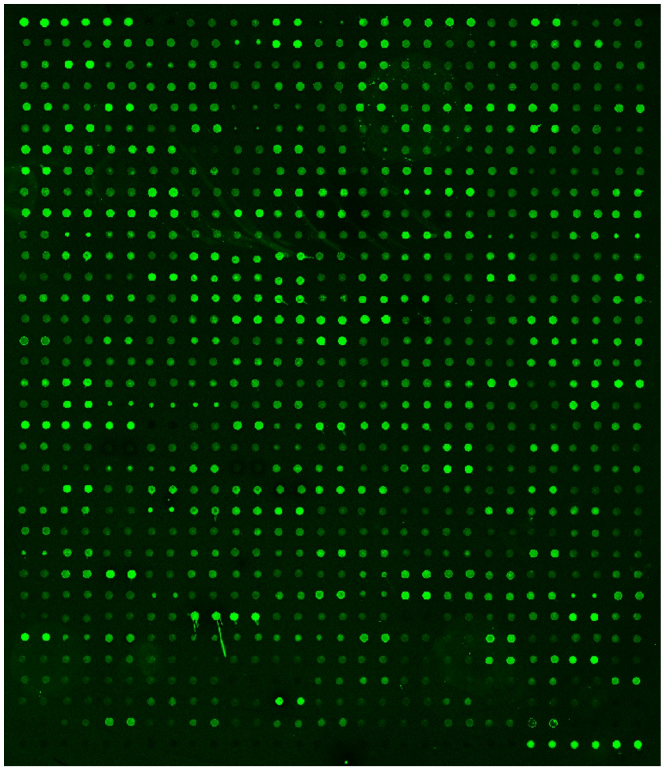
A. Explanation of Controls Spots

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.

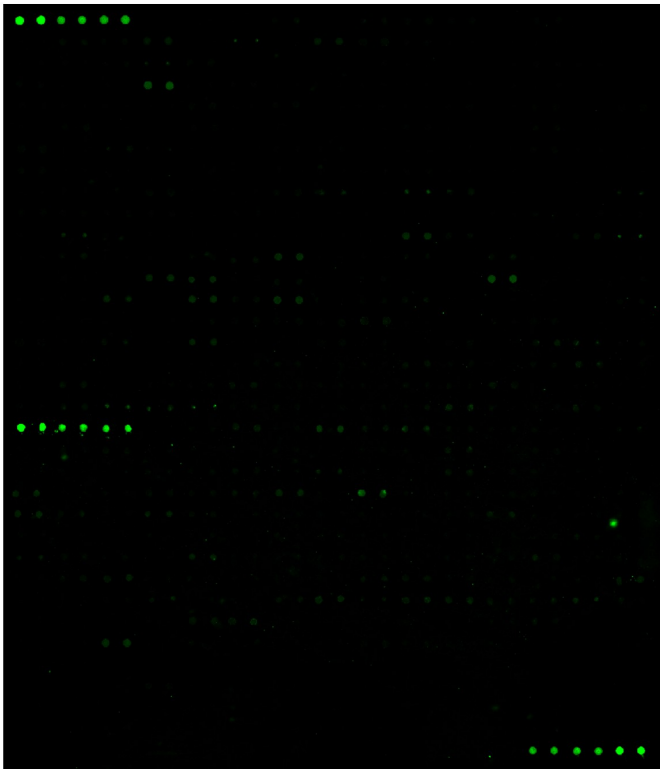
B. Typical Results

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



Buffer Control



Note: In the absence of an external standard curve for each protein detected, there is no means of assessing absolute or relative concentrations of different proteins in the same sample using immunoassays. If you wish to obtain quantitative data (i.e., concentrations of the various analytes in your samples), try using our Quantibody® Arrays as a targeted follow-up experiment.

C. Background Subtraction

Once you have obtained fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Most laser fluorescence scanners' software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEAN signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "F532 Mean - B532"), you may need to subtract the background manually or change the default settings on your scanner's data report menu.

D. Normalization of Array Data

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary. For example, in our Analysis Tool Software (described below), the array represented by data entered in the left-most column each worksheet is the default "reference array."

You can calculate the normalized values as follows:

$X(Ny) = X(y) * P1/P(y)$

Where:
P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is freely available for use with data obtained using RayBio® Biotin Label-based Antibody Arrays. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will

automatically normalize signal intensities to the Positive Controls. Analysis Tool software can be downloaded from the product page on the RayBiotech website.

E. Threshold of Significant Difference

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences in expression levels of each protein between samples or groups.

Any greater than or equal to 1.5-fold increase or less than or equal to 0.65-fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy is around 95%).

VIII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Dilute starting sample less or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completed covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
General	Cross-contamination from neighboring wells	Avoid overflowing wash buffer between wells
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from dying out during experiment

IX. Selected References

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