RayBio® Label-Based (L-Series) Human L14 Array, Glass Slide

Patent Pending Technology
User Manual (Oct 13, 2022)

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

AAH-BLG-14-4 (4 Sample Kit)
AAH-BLG-14-8 (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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TABLE OF CONTENTS

I. Introduction and How It Works..... 3

II. Materials Provided..... 4

A. Storage Recommendations..... 4

B. Additional Materials Required..... 5

III. Overview and General Considerations..... 5

A. Preparation and Storage of Samples..... 5

B. Handling the Glass Slides..... 7

C. Layout of Array Slide..... 8

D. Incubation and Washes..... 9

IV. Protocol..... 10

A. Sample Purification..... 10

B. Biotin Labeling of Sample..... 11

C. Drying of the Glass Slide..... 12

D. Blocking and Incubations..... 12

E. Fluorescence Detection..... 15

V. Antibody Array Map..... 16

VI. Antibody Array Target Lists..... 17

VII. Interpretation of Results..... 18

A. Explanation of Controls Spots..... 18

B. Typical Results..... 18

C. Background Subtraction..... 19

D. Normalization of Array Data..... 19

E. Threshold of Significant Difference..... 20

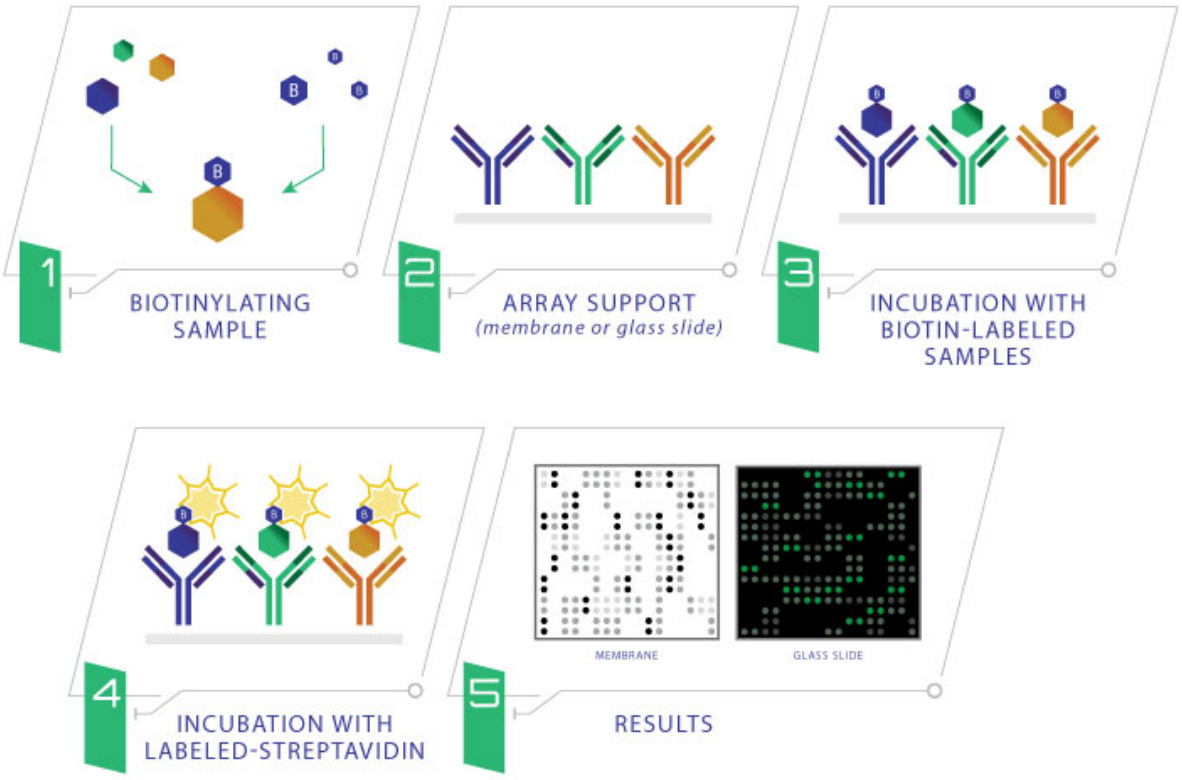
VIII. Troubleshooting Guide..... 21

IX. Selected References.. 22

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.

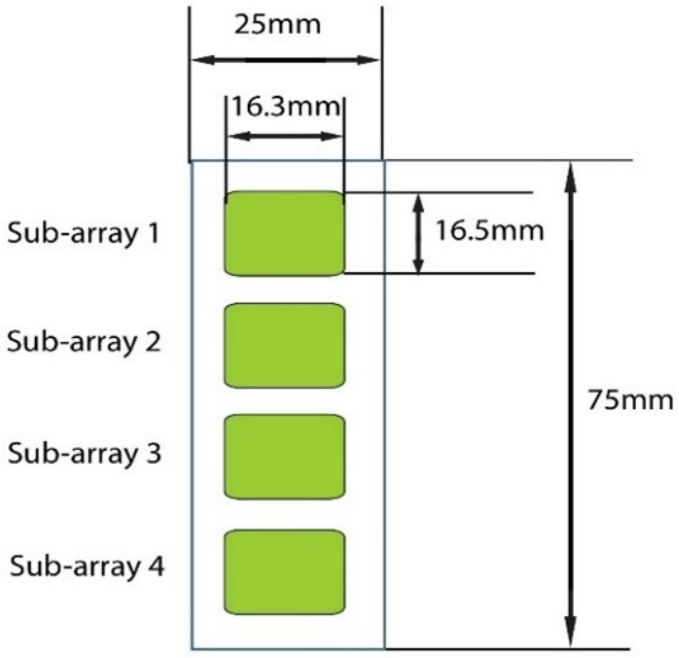
4. Determine the total protein concentration
   For optimal biotin labeling, it is necessary to determine the protein concentration in the cell/tissue lysate. We recommended using a BCA total protein assay (e.g., Pierce, Catalog # 23227).

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

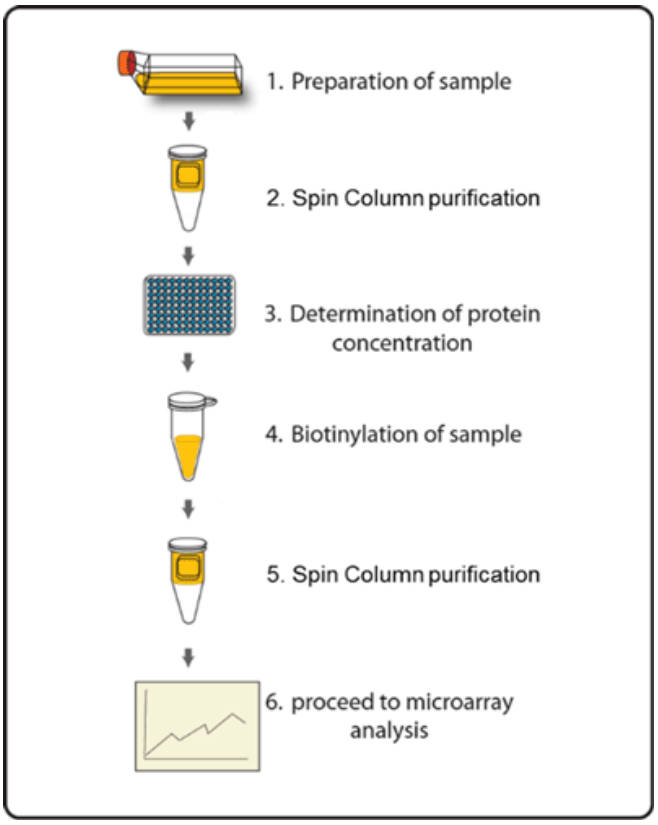
D. Incubations and Washes

- 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.

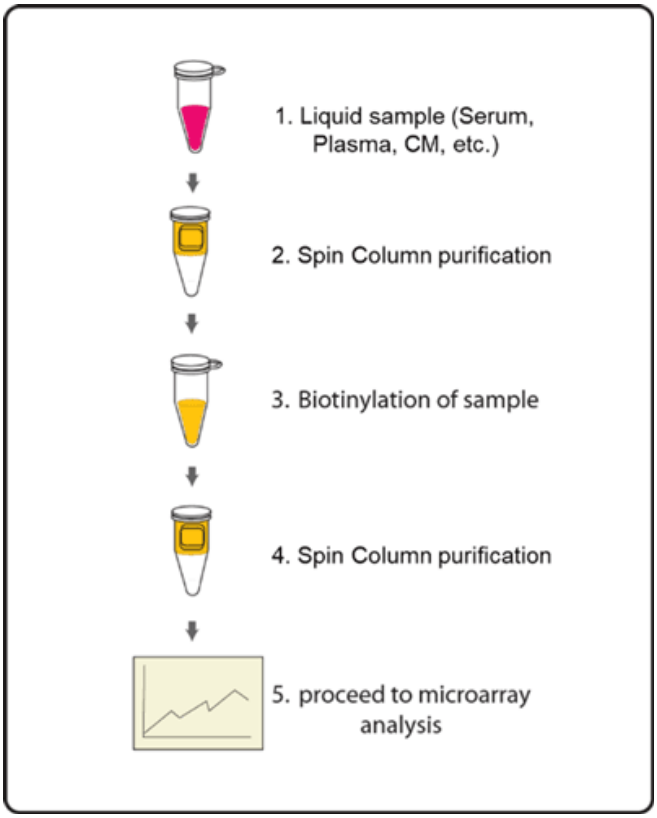
IV. Protocol

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 not 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 completely 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.

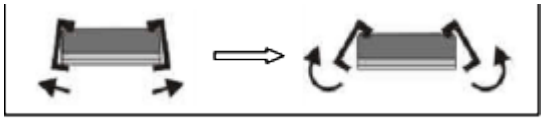
Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.

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.

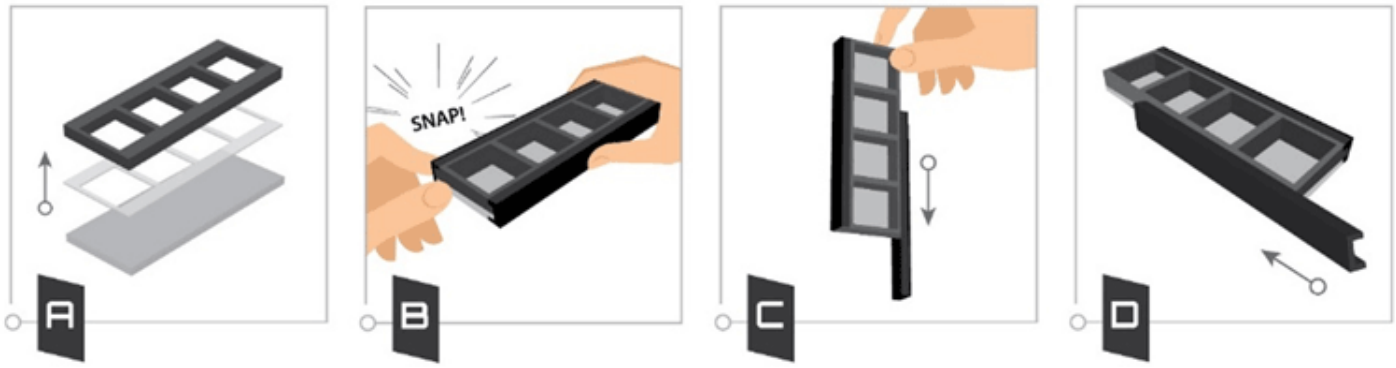
E. Fluorescence Detection

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: Please protect the finished glass slides from temperatures above RT and store them in the dark. 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	ABCA1	73	CLEC1A	145	GCGR	217	KIR2DL4	289	NCOR1	361	PRKCI	433	SPON2
2	ABCG2	74	CLEC2D	146	GHSR	218	KIR2DS1	290	NCOR2	362	PRKDC	434	SQSTM1
3	ACTA2	75	CNDP2	147	GIPR	219	KIR2DS4	291	NDFIP1	363	PRMT1	435	SREBF2
4	ACTN2	76	CNN1	148	GLG1	220	KIR2DS5	292	NEFH	364	PROX1	436	SRPK3
5	ADAM10	77	COL25A1	149	GLI2	221	KIR3DL2	293	NEFL	365	PRR5	437	SSBP1
6	ADAM19	78	COL4A1	150	GLP2R	222	KL	294	NELL1	366	PRSS50	438	STAB1
7	ADAM22	79	COMMD9	151	GLUD2	223	KLK9	295	NETO1	367	PSCA	439	STAT5A
8	ADAM32	80	CORIN	152	GNL3	224	KMO	296	NEU1	368	PSMB10	440	STAT5B
9	ADAMTSL1	81	CPLX1	153	GOLGA2	225	KYNU	297	NEUROG2	369	PSMB9	441	STEAP1
10	ADGRG2	82	CPXM1	154	GOLGB1	226	LAMA3	298	NF2	370	PTCH2	442	STK11
11	AHR	83	CREB1	155	GPC6	227	LEFTY1	299	NFATC2	371	PTGFR	443	STX16
12	AKT1	84	CREG1	156	GPER	228	LGALS2	300	NGFRAP1	372	PTPN22	444	STX1A
13	AKT2	85	CRH	157	GPR35	229	LGR5	301	NINJ2	373	PTPRF	445	SYCP3
14	ALDH2	86	CSE1L	158	GPR50	230	LGR6	302	NKX2-2	374	PTRH1	446	TACC3
15	ALOX5	87	CSNK1A1	159	GPR56	231	LHX5	303	NKX2-5	375	PUM1	447	TACSTD2
16	AMPH	88	CSNK1D	160	GPX2	232	LILRA4	304	NKX6-1	376	PUM2	448	TAF5L
17	ANTXR1	89	CSNK1E	161	GPX4	233	LIMD1	305	NLN	377	PYGO2	449	TAL1
18	ANXA11	90	CSNK1G1	162	GRB7	234	LIPE	306	NLRP10	378	RAB13	450	TANK
19	ANXA4	91	CST2	163	GRM1	235	LITAF	307	NMBR	379	RAB25	451	TBX3
20	APAF1	92	CTSO	164	GRM2	236	LMO2	308	NODAL	380	RAD23A	452	TCF12
21	APBA2	93	CUGBP1	165	GRM8	237	LNPEP	309	NOS2	381	RAD50	453	TDO2
22	ARC	94	CXCR7	166	GSK3B	238	LPAR1	310	NOTCH4	382	RAF1	454	TDRKH
23	ARHGAP1	95	DCC	167	H2AFY	239	LPIN1	311	NOTUM	383	RAMP1	455	TFEB
24	ARSB	96	DDX1	168	HAND2	240	LPIN2	312	NOX4	384	RAMP2	456	TGIF1
25	ATF6	97	DDX4	169	HAO1	241	LRIG2	313	NPHS1	385	RAMP3	457	THRA
26	ATG10	98	DEK	170	HCLS1	242	LRIG3	314	NPY2R	386	RASSF2	458	THRB
27	ATG12	99	DGKG	171	HCRTR2	243	LRPAP1	315	NR1D1	387	RELN	459	TLR9
28	ATG3	100	DKK2	172	HCST	244	LRRC3	316	NR1H2	388	RFC1	460	TMEM173
29	ATG4A	101	DLG4	173	HDAC8	245	LRRTM2	317	NR1I2	389	RFX6	461	TMPRSS11D
30	ATP6AP2	102	DLGAP1	174	HES4	246	LTB4R	318	NR4A2	390	RICTOR	462	TMSB4X
31	ATR	103	DLK2	175	HIPK1	247	LYAR	319	NR6A1	391	RORB	463	TOB1
32	AURKA	104	DLL3	176	HIPK2	248	M6PR	320	NRF1	392	RORC	464	TOP2A
33	AXIN1	105	DLX5	177	HK2	249	MAD2L1	321	NRL	393	RPS6	465	TOPBP1
34	BACE2	106	DNMT3B	178	HMOX2	250	MADCAM1	322	NUAK1	394	RPS6KA1	466	TPSB2
35	BAG1	107	DOCK1	179	HOMER1	251	MAFB	323	OMG	395	RPS6KA2	467	TRAF2
36	BAG6	108	DPP6	180	HOXA1	252	MAGED1	324	ONECUT2	396	RPS6KA3	468	TRAF4
37	BAK1	109	DUSP6	181	HOXB1	253	MAP1LC3A	325	OPRK1	397	RPS6KB1	469	TRAM1
38	BCAR1	110	DYRK1A	182	HRH1	254	MAP2K1	326	OPTC	398	RSPO1	470	TRIM21
39	BCL10	111	EBI3	183	HRH3	255	MAP3K8	327	P2RX7	399	RTN4	471	TRRAP
40	BDKRB2	112	EIF2AK2	184	HS6ST1	256	MAP4K4	328	P2RY10	400	RTN4RL2	472	TXLNA
41	BECN1	113	EIF2S1	185	HSP90B1	257	MAPK4	329	P2RY11	401	RTTN	473	UBE2K
42	BMP10	114	ENPP1	186	HSPA2	258	MAPK6	330	PABPC4	402	RUNX2	474	UBQLN1
43	BRAF	115	EOMES	187	HTR1B	259	MAPK8IP1	331	PAX2	403	RUNX3	475	UCN
44	BTN1A1	116	EP300	188	HTR1D	260	MAPK9	332	PBK	404	RXFP2	476	ULBP2
45	BTNL8	117	EPHA10	189	HTR2B	261	MAPKAP1	333	PCDH15	405	SCD	477	UNC13A
46	CAMK2D	118	ERCC2	190	HTR2C	262	MARCO	334	PCLO	406	SCTR	478	UPF1
47	CAMKK1	119	ERCC3	191	HTR4	263	MBD4	335	PDCD4	407	SDC2	479	USP8
48	CASP10	120	ESRRB	192	HTR6	264	MC5R	336	PDPK1	408	SEMA4B	480	UVRAG
49	CASP7	121	EVI1	193	HTR7	265	MCHR1	337	PEG10	409	SENP1	481	VAMP5
50	CAV1	122	FBXO15	194	ID1	266	MED4	338	PGA5	410	SFRP2	482	VAMP8
51	CCBL1	123	FGF22	195	IFI30	267	MFN2	339	PIAS2	411	SFRP5	483	VAV1
52	CCDC88A	124	FHL2	196	IFITM1	268	MFNG	340	PIAS3	412	SGPL1	484	WAS
53	CCKAR	125	FKBP8	197	IGFBPL1	269	MGAT5	341	PIK3AP1	413	SH2B3	485	WFS1
54	CD151	126	FOLR4	198	IKZF2	270	MIOS	342	PIK3CG	414	SH2D2A	486	WNT16
55	CD1A	127	FOSL1	199	IL11RA	271	MLLT4	343	PIP5K3	415	SH3GLB1	487	WNT3A
56	CD209	128	FOXH1	200	IMPDH2	272	MMP17	344	PITX2	416	SHANK2	488	WNT5A
57	CD274	129	FOXP1	201	INPPL1	273	MOK	345	PKN1	417	SHARPIN	489	WNT7B
58	CD2AP	130	FPR1	202	IRAK3	274	MPL	346	PLK3	418	SIGLEC8	490	WNT8B
59	CD52	131	FPR2	203	IRF5	275	MPP5	347	PLXNA1	419	SIK2	491	XCR1
60	CDC14A	132	FPR3	204	IRS1	276	MRAS	348	PLXNA4	420	SKAP1	492	XPA
61	CDC2	133	FTO	205	ISL2	277	MRE11A	349	PLXNB3	421	SKP2	493	XPNPEP1
62	CDC25A	134	FYB	206	ITGAX	278	MSI1	350	PLXNC1	422	SLC12A7	494	XRCC5
63	CDC25C	135	GAA	207	ITGB1BP2	279	MSI2	351	POLH	423	SLC2A4	495	XRCC6
64	CEACAM4	136	GABARAP	208	ITGB2	280	MUC4	352	POLR1B	424	SMTN	496	YAP1
65	CEBPA	137	GALNT1	209	JAK1	281	MVK	353	POT1	425	SMURF2	497	ZBP1
66	CELSR3	138	GALR1	210	JUB	282	MYB	354	PPM1D	426	SNAI1	498	ZBTB16
67	CETN2	139	GALR2	211	JUND	283	MYF5	355	PPP1CA	427	SNAP23	499	ZIC3
68	CHAD	140	GAP43	212	KATNA1	284	NAALAD2	356	PRDM1	428	SNAP29	500	ZNF281
69	CHD1	141	GAS6	213	KAZALD1	285	NAALADL2	357	PRDX4	429	SOCS2		
70	CHD7	142	GATA6	214	KCNJ2	286	NADK	358	PREB	430	SOCS5		
71	CHL1	143	GBA	215	KEL	287	NCLN	359	PRKACB	431	SOX3		
72	CISH	144	GBL	216	KIR2DL3	288	NCOA2	360	PRKCE	432	SPOCK2		

VII. Interpretation of Results:

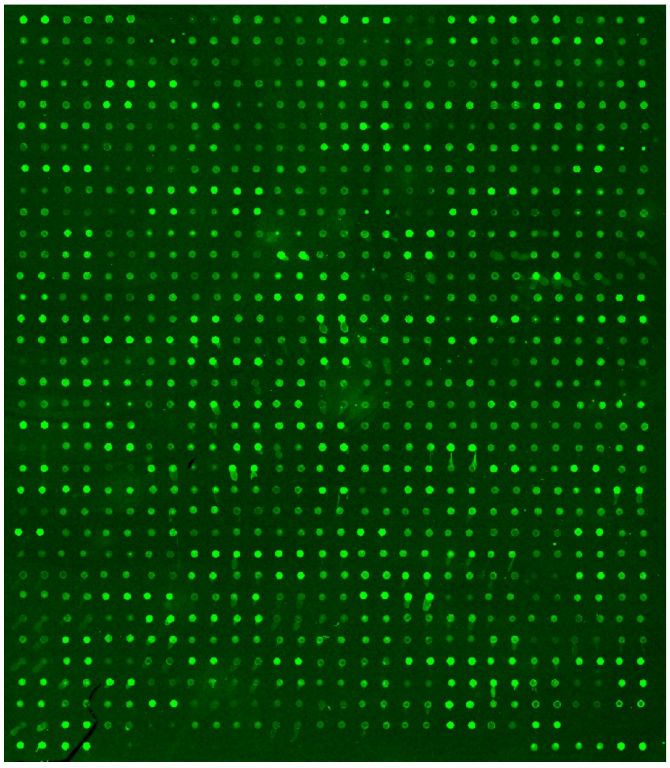
A. Explanation of Controls Spots

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.

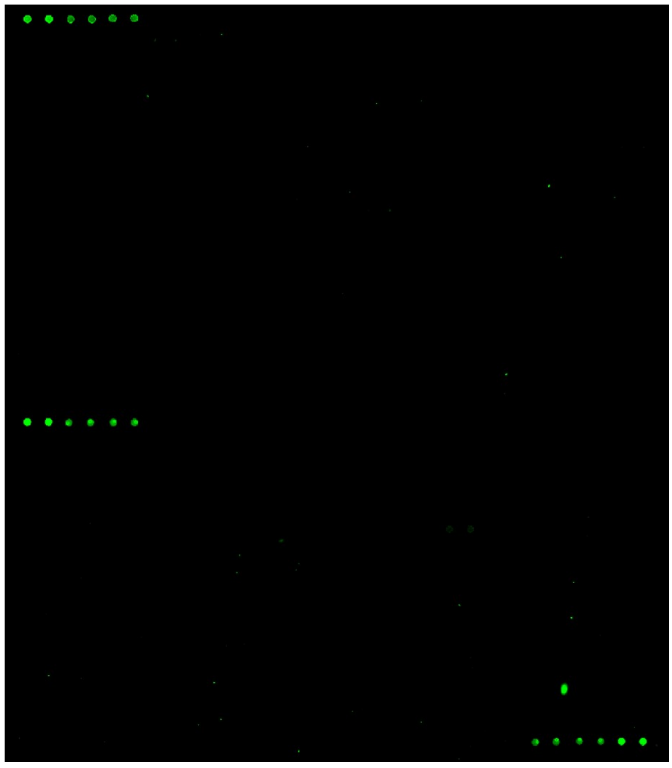
B. Typical Results

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.

Human 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

Christina Scheel et all., *Paracrine and Autocrine Signals Induce and Maintain Mesenchymal and Stem Cell States in the Breast.* Cell. 2011;145, 926-940.

Lin Y, Huang R, Chen L, et al., *Profiling of cytokine expression by biotin-labeled-based protein arrays.* Proteomics. 2003, 3: 1750-1757.

Huang R, Jiang W, Yang J, et al., *A Biotin Label-based Antibody Array for High-content Profiling of Protein Expression.* Cancer Genomics Proteomics. 2010; 7(3):129-141.

Liu T, Xue R, Dong L, et al., *Rapid determination of serological cytokine biomarkers for hepatitis B-virus-related hepatocellulare carcinoma using antibody arrays.* Acta Biochim Biophys Sin. 2011; 43(1):45-51.

Cui J, Chen Y, Chou W-C, et al., *An integrated transcriptomic and computational analysis for biomarker identification in gastric cancer.* Nucl Acids Res. 2011; 39(4):1197-1207.

Jun Zhong et all., *Temporal Profiling of the Secretome during Adipogenesis in Humans.* Journal of Proteome Research. 2010, 9, 5228-5238.

Chowdury UR, Madden BJ, Charlesworth MC, Fautsch MP., *Proteomic Analysis of Human Aqueous Humor.* Invest Ophthalmol Visual Sci. 2010; 51(10):4921-4931.

Wei Y, Cui C, Lainscak M, et al., *Type-specific dysregulation of matrix metalloproteinases and their tissue inhibitors in end-stage heart failure patients: relationship between MMP-10 andLV remodeling.* J Cell Mol Med. 2011; 15(4):773-782.

Kuranda K, Berthon C, Lepêtre F, et al., *Expression of CD34 in hematopoietic cancer cell lines reflects tightly regulated stem/progenitor-like state.* J Cell Biochem. 2011; 112(5):1277-1285.

Toh HC, Wang W-W, Chia WK, et al., *Clinical Benefit of Allogenic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients.* Clin Chem Res. 2009; 15:7726-7736.

Zhen Hou, *Cytokine array analysis of peritoneal fluid between women with endometriosis of different stages and those without endometriosi.* Biomarkers. 2009;14(8): 604-618.

Yao Liang Tang, et al., *Hypoxic Preconditioning Enhances the Benefit of Cardiac Progenitor Cell Therapy for Treatment of Myocardial Infarction by Inducing CXCR4.* Circ Res. 2009;109:197723.

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