

# RayBio® Label-Based (L-Series) Human Calcium Signaling Pathway Screening Array

**Patent Pending Technology**  
**User Manual (Mar 13, 2023)**

For the simultaneous detection of the relative expression of 126 Human proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

**AAH-BLG-CAL-4 (4 Sample Kit)**  
**AAH-BLG-CAL-8 (8 Sample Kit)**

**Please read manual carefully before starting experiment**



**Your Provider of Excellent Protein Array Systems and Services**

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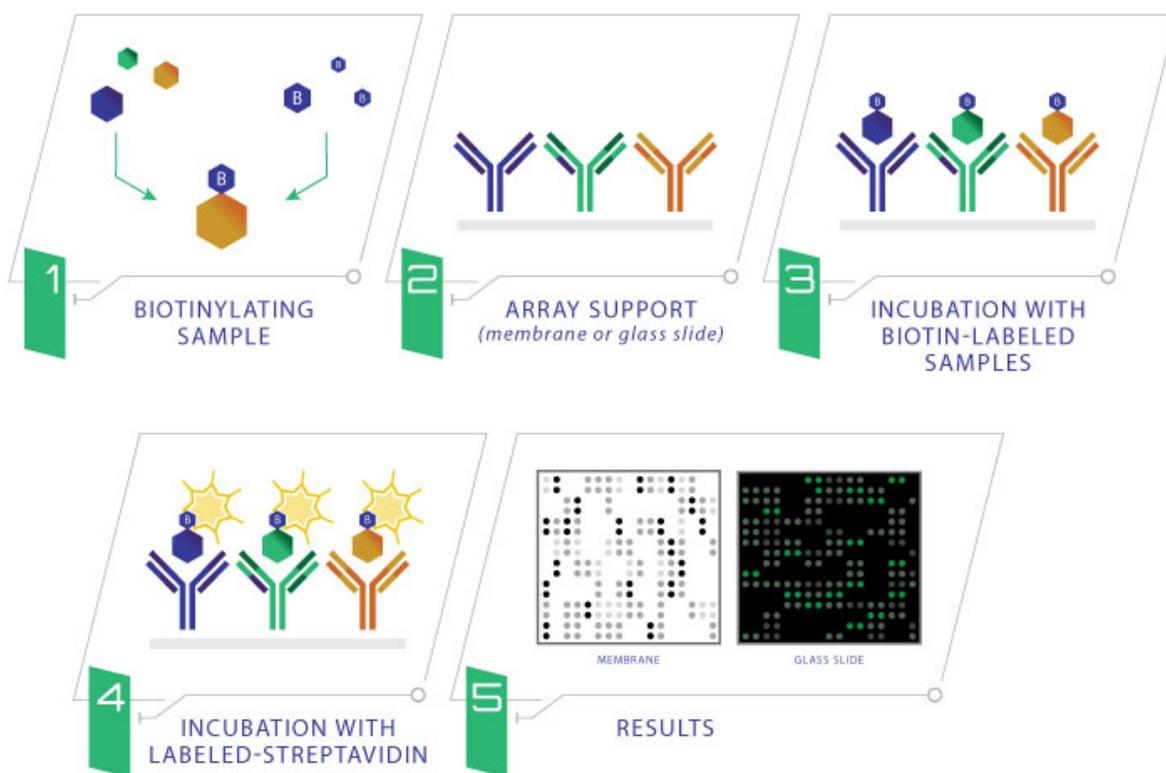
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# I. Introduction

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  $1 \times 10^6$  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<sub>2</sub>O). Solubilize the cells at  $2 \times 10^7$  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<sub>2</sub>O).
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).

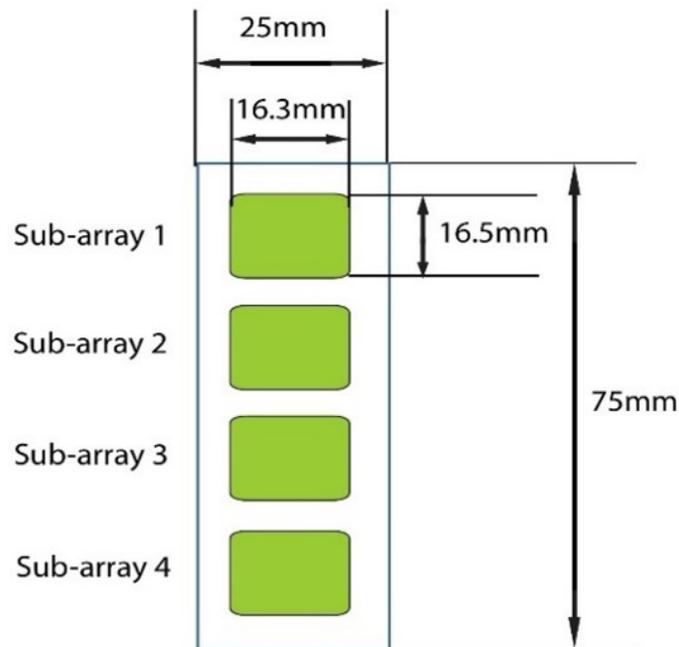
## B. Handling the Glass Slides

- 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

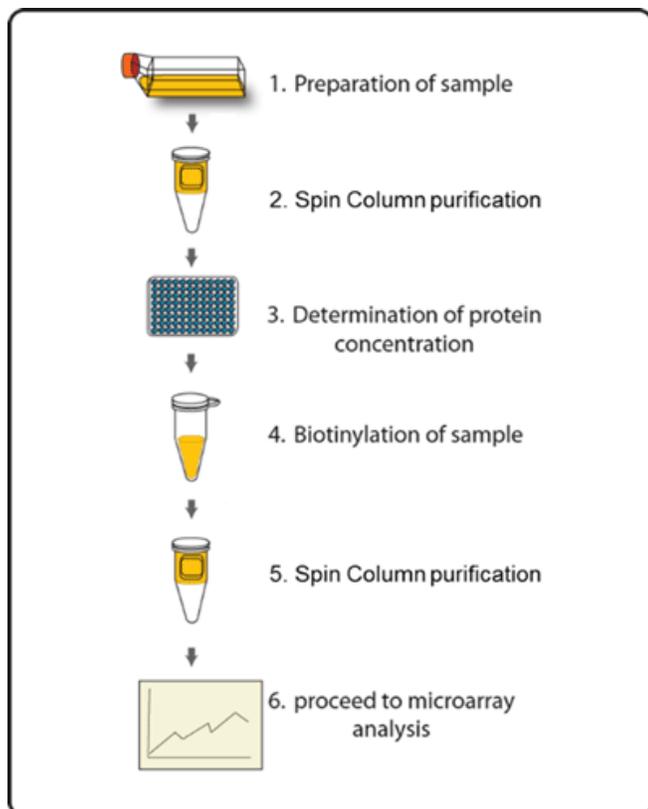
## D. Incubations and Washes

- 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.

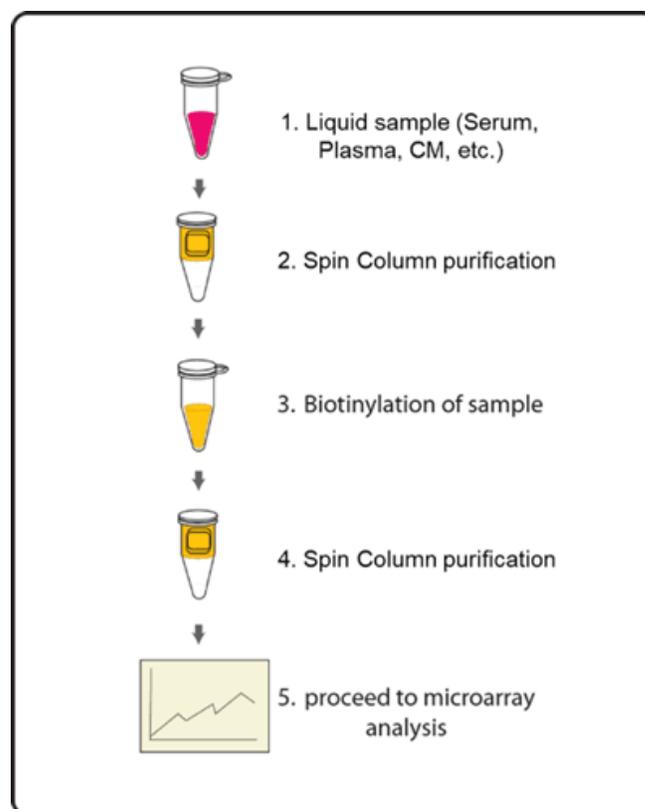
## IV. Protocol

### 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  $\mu$ l 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  $\mu$ l neat supernatant*
- *Serum/Plasma: 2  $\mu$ l serum/plasma in 100  $\mu$ l Labeling Buffer*
- *Cell/tissue lysate: 20  $\mu$ g lysate in 100  $\mu$ l 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  $\mu$ l for each Spin Column. Do not load over 130  $\mu$ l 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  $\mu$ l 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  $\mu$ l of Labeling Reagent into the sample tube (for 120  $\mu$ l supernatant).
  - b. For labeling serum or plasma: Add 8  $\mu$ l of Labeling Reagent into the sample tube (for 2  $\mu$ l serum/plasma in 100  $\mu$ l labeling buffer).
  - c. For labeling cell or tissue lysates: Add 4  $\mu$ l of 1X Labeling Reagent into the sample tube (for 20  $\mu$ g lysate in 100  $\mu$ l labeling buffer).
  - d. For all other body fluid: Add 2  $\mu$ l of Labeling Reagent Solution per 100  $\mu$ g 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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<sub>2</sub>O
14. Decant the samples from each well and wash 3 times with 800  $\mu$ l 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  $\mu$ l 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  $\mu$ l of the concentrated Cy3-Conjugated Streptavidin stock solution into a tube with 800  $\mu$ l of Blocking Buffer. Mix gently.
18. Carefully remove Assembled Glass Slide from container. Remove all of Wash Buffer II from the wells. Add 400  $\mu$ l of 1X Cy3-Conjugated Streptavidin to each sub-array. Cover the incubation chamber with the plastic adhesive strips.

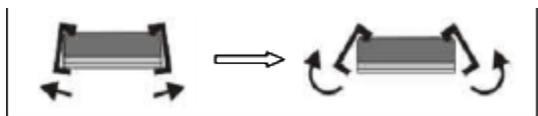
*Note: Avoid exposure to light in Steps 19-25 by covering the Glass Slide Assembly with aluminum foil or incubate in a dark room.*

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<sub>2</sub>O 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 N<sub>2</sub> 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.*

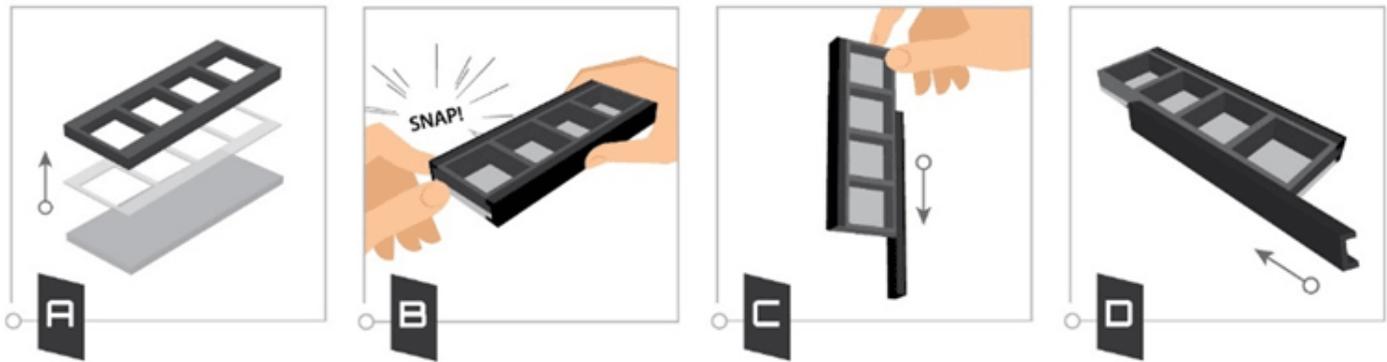
## E. Fluorescence Detection

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

Calcium signaling pathway 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
3	27	27	28	28	29	29	30	30	31	31	32	32	33	33	34	34	35	35	36	36	37	37	38	38	39	39	40	40	41	41
4	42	42	43	43	44	44	45	45	46	46	47	47	48	48	49	49	50	50	51	51	52	52	53	53	54	54	55	55	56	56
5	57	57	58	58	59	59	60	60	61	61	62	62	63	63	64	64	65	65	66	66	67	67	68	68	69	69	70	70	71	71
6	72	72	73	73	74	74	75	75	76	76	77	77	78	78	79	79	80	80	81	81	82	82	83	83	84	84	85	85	86	86
7	87	87	88	88	89	89	90	90	91	91	92	92	93	93	94	94	95	95	96	96	97	97	98	98	99	99	100	100	101	101
8	102	102	103	103	104	104	105	105	106	106	107	107	108	108	109	109	110	110	111	111	112	112	113	113	114	114	115	115	116	116
9	117	117	118	118	119	119	120	120	121	121	122	122	123	123	124	124	125	125	126	126										

## VI. Antibody Array Target List

Calcium signaling pathway array									
Number	Name	Number	Name	Number	Name	Number	Name	Number	Name
1	ADCY1	31	CAMK1	61	GRIN2D	91	PLCD4	121	TACR1
2	ADCY2	32	CAMK2A	62	GRM1	92	PLCG1	122	TACR3
3	ADCY3	33	CAMK2B	63	GRM5	93	PLCG2	123	TNNC1
4	ADCY4	34	CAMK2D	64	HRH1	94	PLCZ1	124	TNNC2
5	ADCY7	35	CAMK4	65	HTR2B	95	PLN	125	VDAC1
6	ADCY8	36	CASQ1	66	HTR2C	96	PPIF	126	VDAC2
7	ADCY9	37	CASQ2	67	HTR4	97	PPP3CA		
8	ADORA2A	38	CCKAR	68	HTR6	98	PPP3CB		
9	ADORA2B	39	CD38	69	HTR7	99	PPP3R1		
10	ADRA1A	40	CHRM1	70	MYLK	100	PPP3R2		
11	ADRA1B	41	CHRM2	71	MYLK4	101	PRKACA		
12	ADRB1	42	CHRM3	72	NOS1	102	PRKACB		
13	ADRB2	43	CHRM5	73	NOS2	103	PRKACG		
14	ADRB3	44	CHRNA7	74	NOS3	104	PRKCA		
15	AGTR1	45	CXCR4	75	ORAI1	105	PRKCB		
16	ASPH	46	DRD1	76	ORAI3	106	PRKCG		
17	ATP2A1	47	DRD5	77	P2RX1	107	PTAFR		
18	ATP2A3	48	EDNRA	78	P2RX5	108	PTGER1		
19	ATP2B1	49	EDNRB	79	P2RX7	109	PTGER3		
20	AVPR1A	50	EGFR	80	PDE1A	110	PTGFR		
21	BDKRB1	51	ERBB2	81	PDE1B	111	PTK2B		
22	BDKRB2	52	ERBB3	82	PDE1C	112	RYR2		
23	CACNA1C	53	ERBB4	83	PDGFRA	113	SLC25A31		
24	CACNA1E	54	F2R	84	PDGFRB	114	SLC25A4		
25	CACNA1G	55	GNA14	85	PLCB1	115	SLC25A5		
26	CACNA1S	56	GNA15	86	PLCB2	116	SLC25A6		
27	CALM1	57	GNAL	87	PLCB3	117	SLC8A1		
28	CALM2	58	GRIN1	88	PLCB4	118	SLC8A3		
29	CALML3	59	GRIN2A	89	PLCD1	119	SPHK1		
30	CALML5	60	GRIN2C	90	PLCD3	120	STIM1		

## VII. Interpretation of Results

### A. Explanation of Controls Spots

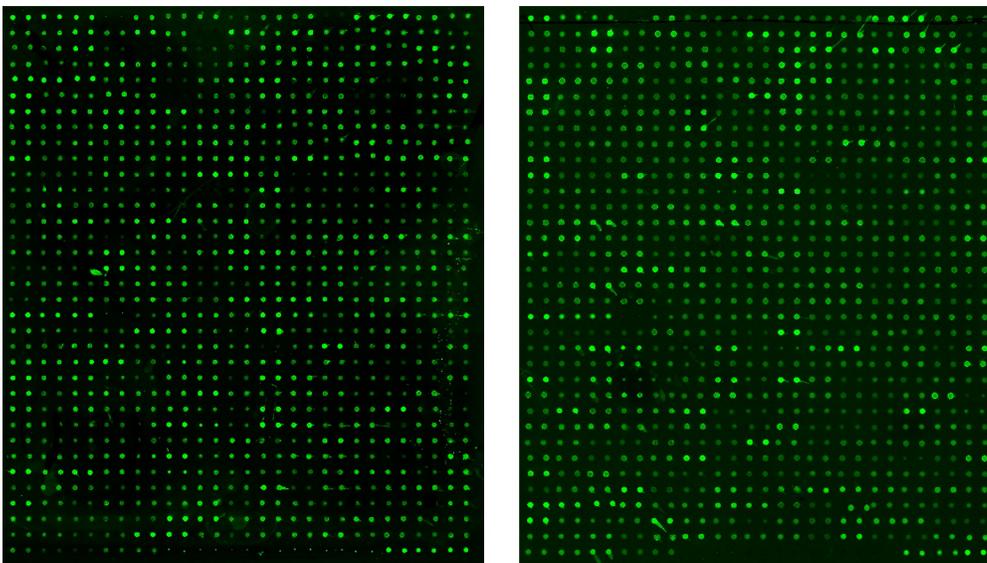
There are three Positive Controls (POS1, POS2, POS3) in each array. These are three levels of standardized biotinylated IgG. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal responses to a known control, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

### B. Typical Results

The following figure shows the typical result of this array probed with sample(s). The images were captured using an Axon GenePix laser scanner. The Positive control signals in the upper left and lower right corners of each array can be used to identify the orientation and help normalize the results between arrays.

Serum

Plasma



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

### C. Background Subtraction

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

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

## D. Normalization of Array Data

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

You can calculate the normalized values as follows:

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

Where:

P1 = mean signal intensity of POS spots on reference array

P(y) = mean signal intensity of POS spots on Array "y"

X(y) = mean signal intensity for spot "X" on Array "y"

X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is freely available for use with data obtained using RayBio® Biotin Label-based Antibody Arrays. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls. Analysis Tool software can be downloaded from the product page on the RayBiotech website.

## E. Threshold of Significant Difference

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

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

## VIII. Troubleshooting Guide

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
<b>Weak Signal</b>	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.
<b>Uneven Signal</b>	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
<b>General</b>	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
<b>High Background</b>	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 drying out during experiment

## IX. Selected References

- Christina Scheel et al., *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 hepatocellular 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 al., *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 and LV 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 endometriosis*. 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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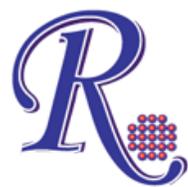
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