

RayBio[®] Human Stem Cell Array 1 (G-Series)

Patent Pending Technology

User Manual (Revised Nov. 24th, 2015)

RayBio[®] Human Stem Cell Array G-Series 1
Cat# AAH-SC-G1-4

RayBio[®] Human Stem Cell Array G-Series 1
Cat# AAH-SC-G1-8

RayBio[®] Human Stem Cell Array G-Series
Testing Service
Cat# AAH-SERV-G

Please read manual carefully
before starting experiment



We provide you with excellent Protein Array systems and services

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

RayBiotech, Inc., the Protein Array Pioneer Company, strives to research and develop new products to meet demands of the biomedical community. RayBiotech's patent-pending technology allows detection of up to 1000 cytokines, chemokines and other proteins in a single experiment. Our format is simple, sensitive, reliable, reproducible and cost-effective.

Our product offerings include:

1. Protein (antigen) Arrays
2. RayBio[®] Cytokine Antibody Arrays
 - C Series (Membrane, chemiluminescence detection)
 - G-Series (Glass chip, fluorescence detection)
3. Pathway- and disease-focused antibody arrays
 - Angiogenesis Antibody Arrays
 - Apoptosis Antibody Arrays
 - Atherosclerosis Antibody Arrays
 - Chemokine Antibody Arrays
 - Growth Factor Antibody Arrays
 - Inflammation Antibody Arrays
 - MMP Antibody Arrays
 - Obesity Antibody Arrays
4. Quantibody[®] Multiplex ELISA Arrays
5. RayBio[®] L-Series Biotin Label-based Antibody Arrays
6. RayBio[®] E-Series Competition-based Antibody Arrays
7. RayBio[®] Phosphorylation Antibody Arrays
 - Receptor Tyrosine Kinases
 - EGFR and ErbB family (site-specific phosphorylation)
8. Over 1,300 different ELISA kits
9. EIA (Competitive ELISA) kits
10. Cell-based Phosphorylation Assay
11. Over 20,000 different antibodies
12. Recombinant proteins
13. Peptide
14. Recombinant antibodies



Protocol for RayBio[®] Human Stem Cell Array G-Series 1

TABLE OF CONTENTS

I.	Introduction.....	1
II.	Product Information.....	5
	A. Storage Recommendations.....	5
	B. RayBio [®] G-Series Glass Chip Layout	6
	C. Materials Provided	6
	D. Additional Materials Required.....	6
	E. How It Works	7
III.	Helpful Tips and General Considerations.....	8
	A. Preparation and Storage of Samples.....	8
	B. Handling Glass Chips	9
	C. Incubations and Washes.....	10
	D. Data Extraction Tips.....	10
IV.	Protocol.....	10
	A. Preparation and Storage of Reagents.....	10
	B. Blocking and Incubations.....	11
	C. Fluorescence Detection.....	14
V.	Interpretation of Results.....	16
	A. Explanation of Control Spots.....	16
	B. Typical Results using G-Series Arrays.....	17
	C. Background Subtraction.....	18
	D. Normalization of Array Data.....	18
	E. Threshold of Significance.....	19
VI.	Antibody Array Map.....	20
VII.	Troubleshooting Guide.....	21
VIII.	Testing Services	23

RayBio[®] Antibody Arrays are patent-pending technology.
RayBio[®] is the trademark of RayBiotech, Inc.

I. Introduction

New techniques such as cDNA microarrays have enabled us to analyze global gene expression¹⁻³. However, almost all cell functions are executed by proteins, which cannot be studied simply through DNA and RNA techniques. Experimental analysis clearly shows disparity can exist between the relative expression levels of mRNA and their corresponding proteins⁴. Therefore, analysis of the proteomic profile is critical.

The conventional approach to analyzing multiple protein expression levels has been to use 2-D SDS-PAGE coupled with mass spectrometry^{5,6}. However, these methods are slow, expensive, labor-intensive and require specialized equipment⁷. Thus, effective study of multiple protein expression levels can be complicated, costly and time-consuming. Moreover, these traditional methods of proteomics are not sensitive enough to detect most cytokines (typically at pg/ml concentrations).

Stem cells are undifferentiated biological cells found primarily in multicellular organisms, that can differentiate into specialized cells and self-renewal to produce more stem cells. Simultaneous detection of multiple stem cell markers undoubtedly provides a powerful tool to study the differentiation mechanisms of human stem cells and to develop disease treatments.

RayBio® Human Stem Cell Array Kit is a rapid, sensitive, and economical tool for the simultaneous detection of the relative levels of expression of 15 stem cell markers. Each antibody was carefully tested using the purified target protein. RayBio® Human Stem Cell Array Kit has several advantages over detection of stem cell markers using single-target ELISA kits or Western blots:

1. More Data, Same or Less Sample: Antibody arrays provide high-content screening using about the same sample volume as traditional ELISA.

2. Global View of Protein Expression: Antibody array screening improves the chances for discovering key factors, disease mechanisms, or biomarkers related to cell signaling.
3. Similar (sometimes better) Sensitivity: As little as 4 pg/ml of MCP-1 can be detected using the C-Series array format. In contrast, our similar MCP-1 ELISA assay has a sensitivity of 40 pg/ml of MCP-1.
4. Wider Detection Range: ELISA typically detects a concentration range of 100 to 1000-fold, while RayBiotech arrays can detect concentration ranges up to 10,000-fold.
5. Better Precision: As determined by densitometry, the inter-array Coefficient of Variation (CV) of spot signal intensities is 5-10%, comparing favorably with ELISA testing (CV = 10-15%).

References

1. Mamlouk O, Balagurumoorthy P, Wang K, Adelstein SJ, Kassis AI. (2012) Bystander effect in tumor cells produced by iodine-125 labeled human lymphocytes. *Int J Radiat Biol*, 88(12):1019-27.
2. Kocaoemer A, Kern S, Kluter H, Bieback K. (2007) Human AB serum and thrombin-activated platelet-rich plasma are suitable alternatives to fetal calf serum for the expansion of mesenchymal stem cells from adipose tissue. *Stem Cells*, 25:1270-1278.
3. Ye Z, Lich JD, Moore CB, Duncan JA, Williams KL, Ting JPY. (2008) ATP Binding by Monarch-1/NLRP12 is critical for its inhibitory function. *Mol Cell Biol*, 28:1841-1850.
4. Sommer G, Kralisch S, Stangl V, Vietzke A, et al. (2009) Secretory products from human adipocytes stimulate proinflammatory cytokine secretion from human endothelial cells. *J Cell Biochem*, 106(4):729-737.
5. Bouazza B, Kratassiouk G, Gjata B, Perie S, et al. (2009) Analysis of growth factor expression in affected and unaffected muscles of

oculo-pharyngeal muscular dystrophy (OPMD) patients: A pilot study. *Neuromusc Disorders*, 19(3):199-206.

6. Dumortier J, Streblow DN, Moses AV, Jacobs JM, et al.(2008) Human Cytomegalovirus Secretome Contains Factors That Induce Angiogenesis and Wound Healing. *J Virol*, 82(13):6524-655.

7. Keren Z, Braun-Moscovici Y, Markovits D, Rozin A, Nahir M, et al. (2009) Depletion of B lymphocytes in rheumatoid arthritis patients modifies IL-8-anti-IL-8 autoantibody network. *Clin Immunol*, 133(1):108-16.

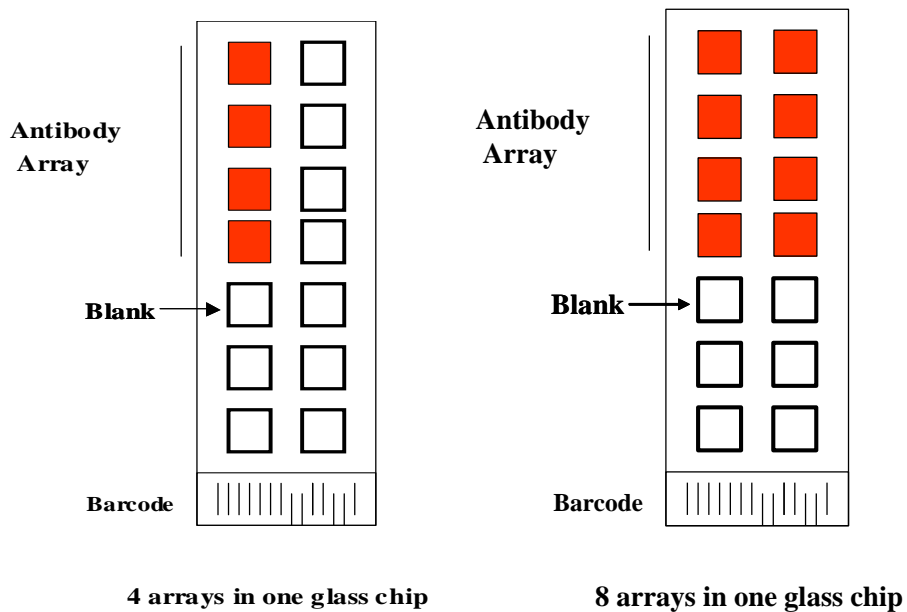
II. Product Information

A. Storage Recommendations:

For best results, we recommend storing the entire kit at -20°C or -80°C upon arrival and using the kit within 6 months of receipt. RayBiotech warrants this product for 6 months if stored in this manner.

Once thawed, store glass chips and 1X Blocking Buffer at -20°C or -80°C and all other component at 4°C . After thawing, the entire kit should be used within 3 months. RayBio[®] Antibody Array kits are robust and will retain full activity even if accidentally stored at room temperature (RT) for up to 24 hours.

B. RayBio[®] G-Series Glass Chip Layout



B. Materials Provided

Item	Description	AAH-SC-G1-4	AAH-SC-G1-8
AAH-SC-G1	RayBio [®] Human Stem Cell G1 Antibody Microarray Glass Chip*	1 chip with 4 Sub-arrays*	1 chip with 8 Sub-arrays*
0103002-HSCG1	Biotin-Conjugated Detection Antibodies	1 ea	2 ea
0103004-H	1,500X HiLyte Plus™ 555 Streptavidin-Fluor†	1 ea	1 ea
0103004-B	1X Blocking Buffer	10 ml	20 ml
0103004-W‡	20X Wash Buffer I ‡	30 ml	30 ml
0103004-W‡	20X Wash Buffer II ‡	30 ml	30 ml
0103004-L	2X Cell Lysis Buffer	10 ml	20 ml
AA-PI	Proteinase Inhibitor	1 vial	2 vials
Other Kit Components: Manual, Adhesive Plastic Strips, 30 ml Centrifuge Tube			

* Kit contains 1 pre-assembled glass chip with either 4 or 8 printed sub-arrays per chip (in sealed plastic envelope)
[NOTE: In some cases, 2 chips x 4 sub-arrays/chip may be substituted in kits containing 8 sub-arrays]

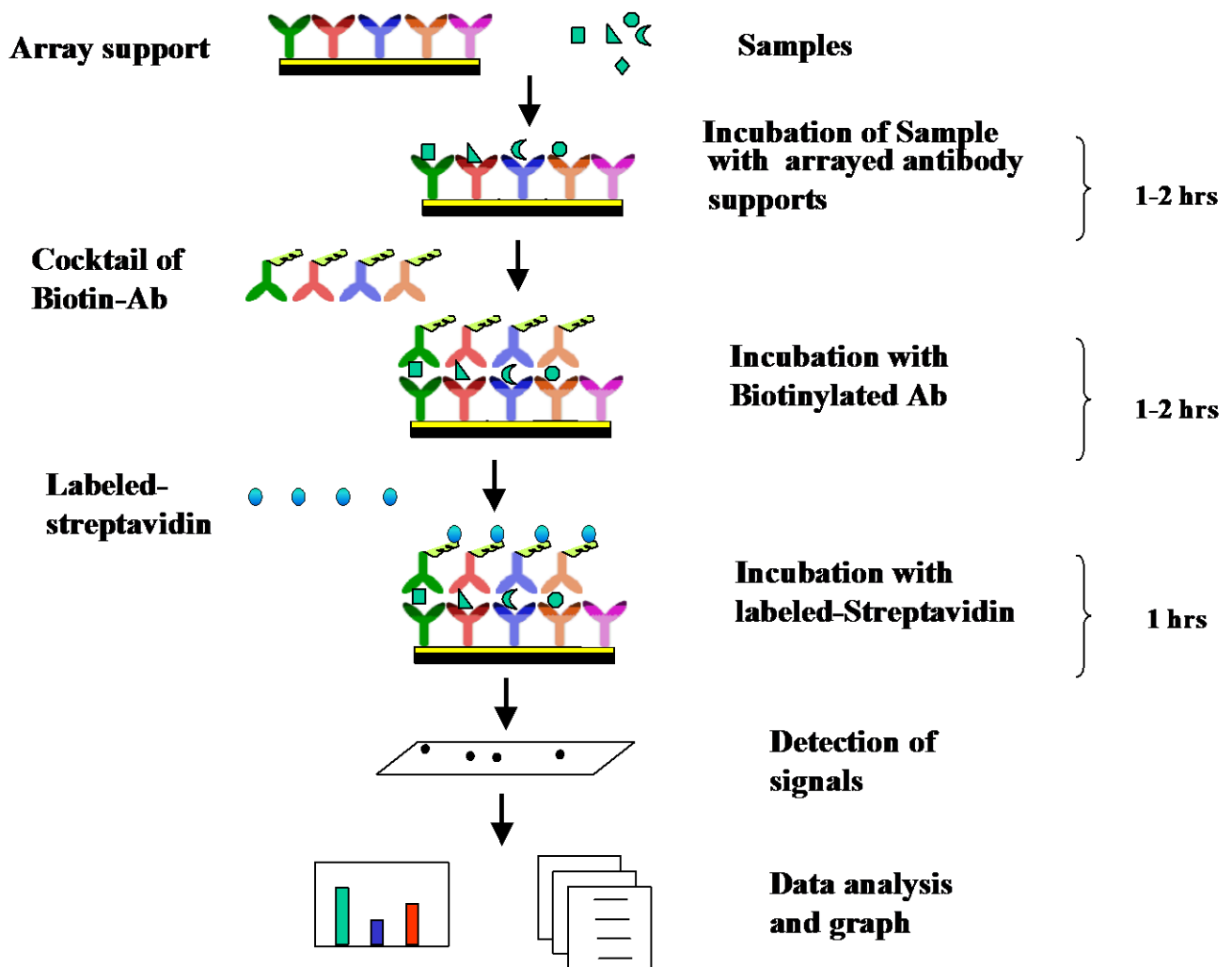
† This fluor is patent-pending technology from Anaspec, Inc.

‡ Wash Buffers are sold as sets

C. Additional Materials Required

- Small plastic boxes or containers
- Pipettors, pipette tips and other common lab consumables
- Orbital shaker or oscillating rocker
- Aluminum foil
- Gene microarray scanner or similar laser fluorescence scanner (see pages 9 & 15)

D. How It Works



III. Helpful Tips and General Considerations

A. Preparation and Storage of Samples

1. General Considerations:

- Freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot your samples prior to initial storage.
- Spin samples hard (5-10 minutes at 10K to 15K RPM) immediately prior to incubation of samples with array.
- Optimal sample concentrations may need to be determined empirically based on the signal intensities of spots and background signals obtained.
- Most samples will not need to be concentrated. If concentration is required, we recommend using a spin-column concentrator with a chilled centrifuge.

2. Sample Preparation

NOTE: All sample dilutions should be made using 1X Blocking Buffer. For all sample types, final sample volume = 50-100 µl per sub-array

The cells can be prepared using the following convention.

For attached cells, remove the supernatant from the cell culture, and wash the cells twice with cold 1X PBS (for cells in suspension, pellet the cells by spinning down at 1500 rpm for 10 min). Make sure to remove any remaining PBS. Then, solubilize the cells at 2×10^7 cells/ml in the 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Pipette up and down to resuspend the cells, and rock the lysates gently at 2-8 °C for 30 min. Transfer the lysates to microcentrifuge tubes and centrifuge at 14,000 x g for 5 min.

It is recommended that sample protein concentrations should be determined using a total protein assay. For incubation with the Antibody Array G-series, use cell lysates at a concentration of 50-

1000 µg/ml (as a starting point, we recommend using 400 µg/ml of cell lysate diluted at least 5-fold with the Blocking Buffer).

Lysates should be used immediately or aliquoted and stored at -70 °C. Thawed lysates should be kept on ice prior to use.

If you experience high background, you may further dilute your sample.

Other Liquid Sample Types: Most often Neat or 2-fold to 5-fold. However, optimal dilutions should be determined empirically.

For tips on sample preparation, please visit our Website:

<http://www.raybiotech.com/Tech-Support/SampleTips.pdf>

B. Handling Glass Chips

- Do not remove glass chip from assembly until Step 16.
- Hold the slides by edges only; do not touch the surface.
- Handle all buffers and slides with powder-free gloves.
- Dry glass chip completely before proceeding to Step 3.
- Handle and dry glass chip in clean environment.
- Avoid breaking glass chip when removing the chamber assembly.

C. Incubations and Washes

- Cover incubation chamber with adhesive film (included in kit) to prevent evaporation, particularly during incubation or wash steps >2 h or with liquid volumes <100 µl per well.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/s).
- Wash steps in Wash Buffer II and all incubation steps may be performed overnight at 4°C.
 - Overnight sample incubations are the most effective at increasing sample spot intensities.
- Avoid cross-contamination of samples to neighboring wells
- To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Chip Assembly to decant, and aspirate the remaining liquid.

- In Wash Steps 6, 12 and 15, you may gently flush wells several times using a wash bottle filled with Wash Buffer I.

D. Scanning and Data Extraction Tips:

For tips on scanning and data extraction, please visit our Website:
<http://www.raybiotech.com/Tech-Support/ScanningTips.pdf>

For a list of recommended scanners, please visit our Website:
http://www.raybiotech.com/files/Tech-Support/Laser_Scanners_for_Glass_Slide_Arrays.pdf.

See also page 18 of this manual.

IV. Protocol

A. Preparation and Storage of Reagents

NOTE: During this protocol, prepare reagents immediately prior to use and keep working dilutions of all reagents on ice at all times.

1. Protease Inhibitor Cocktail: Briefly spin down the Protease Inhibitor Cocktail vial before use. Add 60 μ l of 1X Cell Lysis Buffer to the vial to prepare a 100X Protease Inhibitor Cocktail Concentrate.
2. 2X Cell Lysis Buffer: The 2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water to prepare a 1X Cell Lysis Buffer solution. Then, add 20 μ l of the Protease Inhibitor Cocktail Concentrate into 2 ml of the 1X Cell Lysis Buffer to prepare a 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Mix well before use.
3. Blocking Buffer is supplied at 1X concentration. No dilution is required.
4. Wash Buffers I and II are supplied at 20X concentration.

- a). For each glass chip (4 or 8 sub-arrays/chip), dilute 5 ml of 20X concentrate with deionized H₂O to a final volume of 100 ml each of Wash Buffer I & Wash Buffer II.
 - b). Wash buffer reagents at working dilution (1X) can be stored at 4°C for up to 1 month. Stock solutions at 20X can be stored 4°C for up to 3 months.
5. Biotin-conjugated Anti-Cytokines are supplied at high concentration in a small liquid bead (typically ~2-5 µl).
- a). Spin down the tube prior to reconstitution, as the concentrated liquid bead may have moved to the top of the tube during handling.
 - b). Prepare stock reagent by adding 300 µl 1X Blocking Buffer to Biotin-Conjugated Anti-Cytokines. Mix well.
 - c). 1X Biotin-Conjugated Anti-Cytokines may be stored for 2-3 days at 4°C.
6. Streptavidin-Fluor is supplied at 1500x concentration.
- a). Mix the tube containing 1500X Streptavidin-Fluor well before use, as precipitants may form during storage.
 - b). Add 100 µl of 1X Blocking Buffer to tube containing 1500X Streptavidin-Fluor. Mix well.
 - c). Quantitatively transfer all of Streptavidin-Fluor reagent from the original tube to a larger one, and dilute with 1X Blocking Buffer to a final volume of 1500 µl (ie, 1.5 ml).
 - d). This working dilution can be stored for 3-5 days at 4°C.

B. Blocking and Incubations

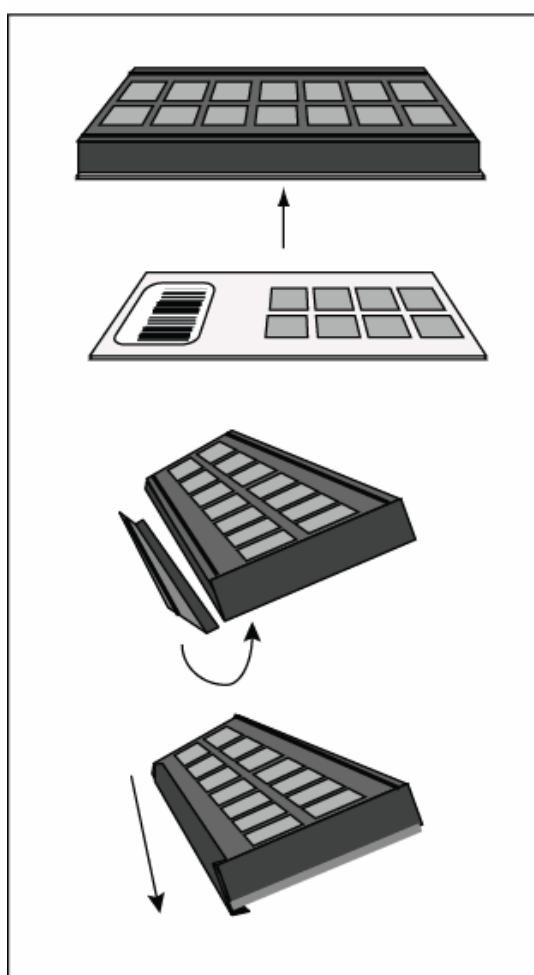
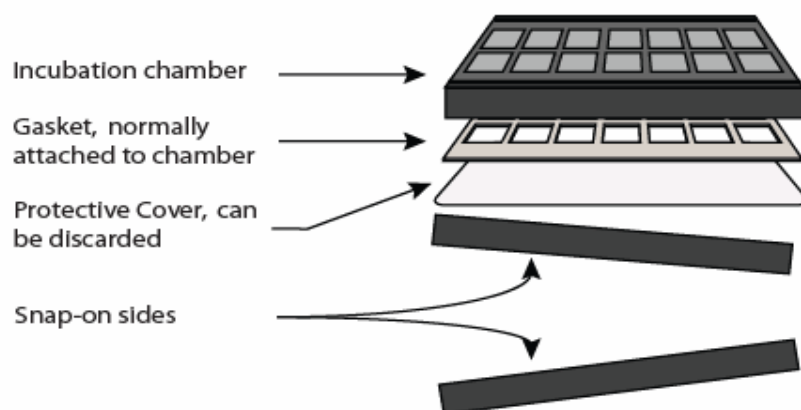
NOTE: Please carefully read Section III of this manual before proceeding

NOTE: Prepare all reagents immediately prior to use as described above (Section IV.A) and before proceeding.

- 1) Remove the package containing the glass chip assembly from the freezer. Place unopened package on the benchtop and allow the glass chip assembly to equilibrate to room

Instructions for incubation chamber assembly

G Series and Quantibody Arrays



1 Carefully place slide at bottom of the chamber as shown. The slide will adhere somewhat to the bottom. Warning: the slide is fragile, so do not apply more than gentle force to the apparatus.

2 While gently holding chamber and slide, place side on chamber as shown, beginning with bottom flap first.

3 Then, press the top of the side into groove on chamber, and then apply even, gentle pressure from one end to the other. Repeat this procedure with the other side.

temperature (RT), approx. 15 min. Open package, remove the glass chip assembly and place in laminar flow hood to dry for 1-2 hours.

NOTE: Be sure glass chip is completely dry before proceeding.

2) If necessary, assemble the glass chip into incubation chamber and frame as shown on page 12. (Note: if you slide is already assembled, you can proceed directly to Step 3).

3) Add 100 μ l 1 X Blocking Buffer into each well and incubate at RT for 30 min to block slides.

NOTE: Only add reagents or samples to wells printed with antibodies (see diagram on page 5)

4) Decant Blocking Buffer; then aspirate remaining liquid from each well.

NOTE: To aspirate liquid samples or reagents from wells, gently place the pipette tip only in the corners of the well. Do not scrape the pipette tip across the surface of the chip.

5) Add 50 to 100 μ l of each sample to each sub-array. Cover the incubation chamber with Adhesive film (included in kit). Incubate arrays with sample at room temperature for 1 to 2 hours. Dilute sample using 1X Blocking Buffer if necessary.

6) Remove adhesive film, and carefully aspirate samples from sub-arrays, touching only the corners with your pipette tip.

7) Wash 3 x 2 min with 150 μ l Wash Buffer I at RT. Be sure to completely remove sample and Wash Buffer each time and use fresh buffer for each wash. Decant final wash solution before proceeding to next step.

NOTE: Try to prevent solution from flowing into neighboring wells.

8) Obtain a clean container (eg, pipette tip box or slide staining jar) and place glass chip assembly into the container. Add enough 1X Wash Buffer I to submerge the entire glass chip with frame intact (approx. 30-50 ml) and remove all bubbles in wells. Wash 10 min at RT with gentle rocking or shaking.

- 9) Remove assembled glass chip from container and invert to decant liquid. Decant buffer from container and replenish with 1X Wash Buffer I. Submerge the entire glass chip assembly and wash 10 min at RT with gentle rocking or shaking.
- 10) Remove assembled glass chip from container and invert to decant liquid. Decant buffer from container and repeat Steps 8 & 9 with Wash Buffer II.
- 11) Remove assembled glass chip from container and invert to decant liquid, then carefully aspirate wash buffer from wells, touching only the corners with your pipette tip.
- 12) Add 70 μ l of 1X Biotin-conjugated Anti-Cytokines to each sub-array. Cover incubation chamber with Adhesive film (included in kit). Incubate at RT for 2 hours with gentle rocking or shaking.
- 13) Carefully aspirate Biotin-conjugated Anti-Cytokine reagent. Wash as described in Step 7 above, first with Wash Buffer I then with Wash Buffer II, making sure to completely remove buffer between washes and after final wash.
- 14) Add 70 μ l of 1X Streptavidin-Fluor to each sub-array. Cover the incubation chamber with Adhesive film (included in kit), then cover entire assembly with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at RT for 2 hours with gentle rocking or shaking.
- 15) Remove aluminum foil and adhesive film. Carefully aspirate the Streptavidin-Fluor reagent. Wash as described in Step 7 above, first with Wash Buffer I then with Wash Buffer II, making sure to completely remove buffer between washes and after final wash.
- 16) Remove the glass chip from the frame assembly. Place the whole chip in 30 ml centrifuge tube provided, or slide staining jar. Add enough Wash Buffer I to cover the whole slide (about 20 ml) and gently rock or shake at RT for 10 min.

- 17) Decant buffer and repeat wash as described in Step 16 (1 x 10 min with Wash Buffer I).
- 18) Decant buffer and repeat wash as described in Step 16, but this time using Wash Buffer II for only 2-3 minutes.
- 19) Decant buffer, remove the glass chip from the tube, then gently rinse the slide with de-ionized H₂O using a plastic wash bottle.
- 20) Remove water droplets by applying suction gently with a pipette tip.

NOTE: Be careful not to touch the array portions of the slide with your pipette tip, only touch the sides of the slide.

C. Obtaining Fluorescent Signal Intensities:

- 21) Allow glass chip to dry in a laminar flow hood for 20 minutes or until slide is completely dry. Place chip under an aluminum foil tent to protect it from light. Make sure the slides are absolutely dry before scanning or storage.

Note: Unlike most Cy3 fluors, the HiLyte Plus™ Fluor 555 used in this kit is very stable at RT and resistant to photobleaching on completed glass chips. However, please protect glass chips from strong light and temperatures above RT.

- 22) You may proceed immediately to scanning (Step 23), or you may store the slide at RT and to scan at a later time.
- 23) Scan the glass chip with a laser scanner (such as Innopsys' InnoScan®) using cy3 or "green" channel (excitation frequency = 532 nm). For tips on scanning, visit our Website: <http://www.raybiotech.com/Tech-Support/ScanningTips.pdf>

NOTE: If you do not have a laser scanner, for a nominal fee you can send your slide to us for scanning and data extraction using Innopsys' InnoScan, and we will return the results to you. Using

using alternate protocols, RayBio® G-Series arrays are also compatible with Li-Cor's Odyssey and other microarray scanners.

V. Interpretation of Results:

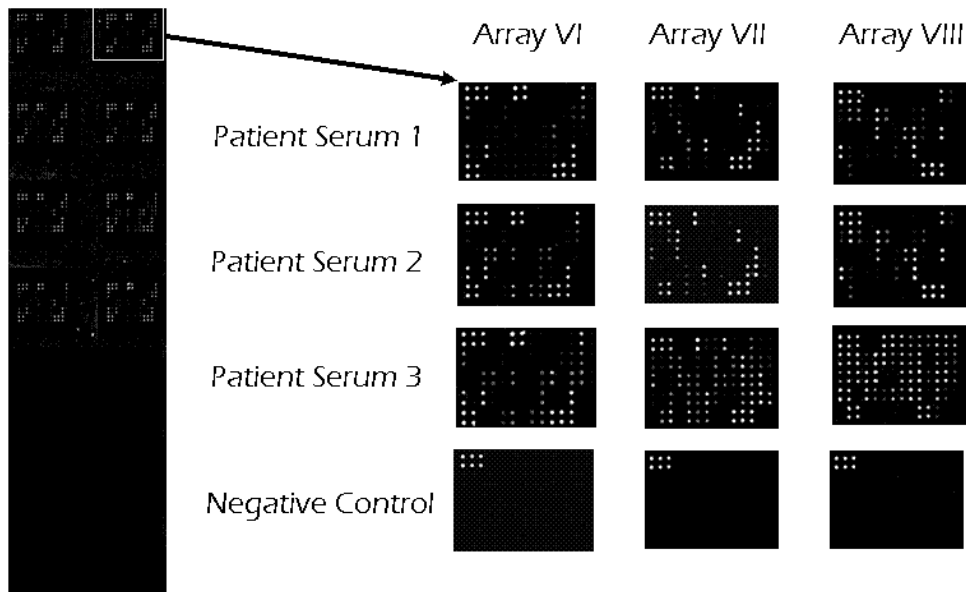
A. Explanation of Controls Spots

Positive Controls (POS1, POS2, POS3) are equal amounts of biotinylated IgGs printed directly onto the array. 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.

Negative Control (NEG) spots are a protein-containing buffer (used to dilute antibodies printed on the array). Their signal intensities represent non-specific binding of Biotin-conjugated anti-Cytokines and/or Streptavidin-Fluor. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical results from RayBio® G-Series Antibody Arrays

The following figure shows typical results obtained using RayBio® Antibody Array G-Series Arrays. The images were captured using a GenePix 4000B scanner.



In this example, sera from several patients were incubated with Human Cytokine Arrays 6, 7 & 8, (sold together as Human Cytokine Array G-Series 2000, AAH-CYT-G2000-4 or AAH-CTY-G2000-8) and processed using this standard protocol.

The 6 strong signals of the Positive Control spots in the upper-left corner are useful for proper orientation of the array image.

If scanned using optimal scan settings, 3 distinct Positive Control signal intensities will be seen: POS1>POS2>POS3. If all of these signals are of similar intensity, try increasing or decreasing laser power and/or signal gain settings.

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

C. Background Subtraction:

Most laser fluorescence scanner software have an option to automatically measure the local background around each spot. As with spot signal intensities, we recommend using MEDIAN background signals. If your resulting fluorescence signal intensity

reports do not include these values (eg, a column labeled as “MED532-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 can be arbitrary. For example, in our Analysis Tool Software, 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 available for use with data obtained using RayBio[®] G-Series 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.

To order the Analysis Tool, please contact us at +1-770-729-2992 or info@raybiotech.com for more information.

E. Threshold of significant difference in expression:

After subtracting background signals and normalization to Positive controls, comparison of signal intensities for antigen-specific antibody spots between and among array images can be used to

determine relative differences in expression levels of each analyte (ie, protein detected) between samples or groups.

Any ≥ 1.5 -fold increase or ≤ 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 $\approx 95\%$).

NOTE: In the absence of an external standard curve for each analyte, there is no means of assessing absolute or relative concentrations of different analytes in the same sample using immunoassays. If you wish to obtain quantitative data (ie, concentrations of the various analytes in your samples), try using our Quantibody® Multiplex ELISA arrays instead.

Data Extraction Tips:

- Ignore any comet tails
- Define the area for signal capture for all spots as 110-120 micron diameter, using the same area for every spot.
- Use median signal value, not the total or the mean
- Use local background correction (also median value).
- Exclude obvious outlier data in its calculations.
- Scan all slides at same PMT

VI. RayBio® Human Stem Cell Array G-Series 1 Map:

Detects 15 human stem cell markers in one experiment

Each antibody is spotted in duplicate vertically		A	B	C	D	E	F	G	H
	1	POS1	POS2	POS3	NEG	Alpha- fetoprotein	OCT-4	BMPR-1A	ALK-6
	2								
	3	Brachyury	CD38	E-Cadherin	GATA4	hCG beta	Nanog	Nestin	PDX-1
	4								
	5	SOX2	SOX17	VEGFR2	NEG	NEG	NEG	NEG	POS2
6									

VII. Troubleshooting guide

Problem	Cause	Recommendation
No signal for any spots, including Positive Controls	Global detection failure	Adjust scanner settings or re-assemble chip into holder, wash slide 2 x 5 min with 150 μ L Wash Buffer II and repeat Steps 12-21.
Similar signal intensities for POS1/2/3	Improper laser power and/or PMT setting	Repeat scan using higher and/or lower laser power or PMT settings
High background signals	Incomplete washes	Carefully follow wash protocols, and/or increase wash times
	Sample concentration is too high	Repeat using lower sample concentration
	Fluor and/or Anti-Cytokines are too concentrated	Review protocol for dilution of reagents
Uneven background and/or missing spots	Bubbles present on chip during incubations	Be sure to completely remove all bubbles from chip surface
	Evaporation during incubation steps	Cover chamber assembly during washes and incubations
	Pooling/precipitation of sample or reagent; Incomplete washes.	Cover chamber assembly and use a rocker or shaker during washes and incubations; carefully follow wash protocols.
	Sample is too concentrated	Repeat experiment using more dilute sample
Randomly scattered high-intensity spots	Dust or other particulates	Dry slides in laminar flow hood and/or use clean containers and powder-free gloves.

Weak or no signals antigen-specific pots + Low Background	Sample is too dilute	Repeat experiment using higher sample concentration
	Improper dilution of Anti-Cytokines or Streptavidin-Fluor	Re-assemble chip into holder, wash 2 x 5 min with 150 μ L Wash Buffer II and repeat Steps 12-21. Spin down reagents before diluting and mix well.
	Other Tips	Rescan at higher laser power or signal gain setting
		Repeat using higher sample concentration and/or incubate with sample O/N at 4°C
		Increase concentration of and/or length of incubation with Biotin-conjugated Anti-Cytokine (+ add'l large volume wash following Biotin-Ab incubation)
		Review proper storage conditions for kit components

Testing Services:

RayBiotech offers full testing services using any of our Array, ELISA or EIA products, including customized products.

Just send your samples, and we will send you the results.

Custom Services:

1. Customized Antibody and Protein Arrays
2. Customized Phosphorylation Arrays
3. Peptide synthesis
4. Peptide arrays
5. Recombinant protein and antibody production
6. ELISA
7. EIA
8. Assay development

Technology Transfer Program:

Have you developed technologies or reagents of interest to the scientific and research community? RayBiotech can help you commercialize your technologies, reagents and dream.

RayBio[®] Cytokine Antibody Arrays are patent-pending technology developed by RayBiotech.

This product is intended *for research only* and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for 6 months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

RayBio[®] is a registered trademark of RayBiotech, Inc.

HiLyte Plus[™] is a trademark of Anaspec, Inc.

InnoScan[®] is a registered trademark of Innopsys, Inc.

This product is for research use only.



©2015 RayBiotech, Inc.