

# RayBio<sup>®</sup> Human Autoimmune Disease IgG Autoantibody Detection Array G1

Glass–chip-based protein arrays

**User Manual**

(Revised June 7, 2017)

**For simultaneous detection of  
33 human autoantibodies (IgG type)  
from autoimmune disease patients' sera**

**Human Autoimmune Disease IgG Autoantibody Detection Array G1  
(Cat #. PAH-AIDG-G1)**

**Human Autoimmune Disease IgG Autoantibody Detection Array G1 Service  
(Cat #. PAH-AIDG-G1-SERV)**

**Please read manual carefully before starting experiment**

**For research use only. Not for diagnostic or therapeutic use.**



**We Provide You with Excellent  
Protein Array Systems and Service**

---

**Tel:** 1-888-494-8555, 770-729-2992

**Fax:** 1-770-206-2393

**Website:** [www.raybiotech.com](http://www.raybiotech.com)

**E-mail:** [info@raybiotech.com](mailto:info@raybiotech.com)

## ***Array Overview***

|                                   |  |
|-----------------------------------|--|
| <b>Autoimmunogen Detected</b>     | 33 targets   |
| <b>Array Format</b>               | Glass slide  |
| <b>Autoantibody Type Detected</b> | Human IgG  |
| <b>Array Size</b>                 | Detection of 16 samples per glass slide              |
| <b>Detection Method</b>           | Fluorescence (Cy3 equivalent dye) with laser scanner |
| <b>Sample Volume</b>              | ~ 2 $\mu$ L serum per array                          |
| <b>Assay Duration</b>             | < 8 hours  |

### **Additional Custom Protein Array Services We Provide:**

We also offer a completely customized array service with many options that can be requested by a customer.

#### **1. Experimental Design**

RayBiotech's protein array experts can assist you in your experimental design based on your project purpose.

#### **2. Customized Arrays**

- Select your targets from our array protein lists.
- Send your targets to us, such as proteins, synthesized polypeptides, DNA, and any other molecules.
- If your targets are not available on the market, we can produce recombinant proteins using our rapid bacterial gene expression system or mammalian cell gene expression system. For small polypeptides, we also provide peptide synthesis service.

#### **3. Full Testing Services**

You can send your samples to us, and our expert staff will run the experiments and provide you with the fully analyzed results.

# TABLE OF CONTENTS

| <u>Section</u>  | <u>Page</u> |
|---|-------------|
| <b>I. Kit Contents and Storage</b> .....                  | <b>4</b>    |
| 1. Array Kit Components.....                              | 4           |
| 2. Storage.....   | 5           |
| 3. Additional Materials Required.....                     | 5           |
| <b>II. Introduction</b> .....                             | <b>6</b>    |
| 1. Principle of the Assay.....                            | 6           |
| 2. Array Features.....                                    | 7           |
| <b>III. Overview and General Considerations</b> .....     | <b>9</b>    |
| 1. Sample Collection, Preparation, and Storage.....       | 9           |
| 2. Handling of Glass Arrays.....                          | 9           |
| 3. Incubation.....  | 10          |
| 4. Layout of Glass Arrays.....                            | 10          |
| 5. Incubation Chamber Disassembly .....                   | 11          |
| 6. Incubation Chamber Assembly .....                      | 11          |
| <b>IV. Protocol</b> .....                                 | <b>12</b>   |
| 1. Blocking and Sample Incubation.....                    | 13          |
| 2. Biotin-conjugated Anti-human IgG Incubation .....      | 14          |
| 3. Cy3-conjugated Streptavidin Incubation.....            | 14          |
| 4. Fluorescence Detection.....                            | 15          |
| <b>V. Data Analysis</b> .....                             | <b>17</b>   |
| 1. Data Extraction.....                                   | 17          |
| 2. Control Systems.....                                   | 17          |
| 3. Data Normalization.....                                | 17          |
| 4. Threshold of Significant Difference in Expression..... | 18          |
| 5. RayBio® Analysis Tool.....                             | 18          |
| 6. Interpretation of Results.....                         | 19          |
| <b>VI. Appendix</b> .....                                 | <b>21</b>   |
| 1. Array Map.....   | 21          |
| 2. Array List.....  | 22          |
| 3. Troubleshooting Guide.....                             | 23          |
| 4. Reference List.....                                    | 24          |
| 5. Quick Protocol.....                                    | 25          |
| 6. Experiment Record Form.....                            | 26          |

## I. Kit Contents and Storage

This manual provides the guidelines and instructions for detecting IgG type autoantibodies from autoimmune disease patient's serum or other fluids using the RayBio® Human Autoimmune Disease IgG Autoantibody Detection Array Kit.

### 1. Array Kit Components

Each array kit (16 sample kit) contains the following components:

| Item     | Description   | Cat #.<br>PAH-AIDG-G1-16  |
|----------|---|---|
| <b>A</b> | RayBio® Autoimmune Disease IgG Autoantibody Detection Array. <b>Assembled Glass Slide</b> | 1 slide<br>(16 sub-arrays)  |
| <b>B</b> | <b>1,000x Biotin-conjugated Anti-Human IgG</b><br>1.5 µl/vial                             | 2 vials   |
| <b>C</b> | <b>1,500x HiLyte 555 Streptavidin</b><br>1 µl/vial  | 2 vials   |
| <b>D</b> | <b>Blocking Buffer</b>  | 1 bottle  |
| <b>E</b> | <b>20x Wash Buffer I</b>  | 1 bottle  |
| <b>F</b> | <b>20x Wash Buffer II</b>   | 1 bottle  |
| <b>G</b> | <b>Adhesive Plastic Strips</b>  | 1 strip   |
| <b>H</b> | <b>30 ml-Centrifuge Tube</b>  | 1 tube  |
| <b>I</b> | <b>User Manual</b>  | Please download from<br><a href="http://www.raybiotech.com/">http://www.raybiotech.com/</a> |
| <b>J</b> | <b>Array Target List</b>  |   |
| <b>K</b> | <b>Array Map</b>  |   |

#### Notes:

- *Items B & C:* spin down and dilute with Blocking Buffer (*Item D*) prior to use.
- *Items E & F:* dilute with distilled water prior to use.

## **2. Storage**

Upon arrival, all components of array kit should be immediately stored at -20 °C to -80 °C until just before the experiment. At -20 °C to -80 °C, the kit will retain complete activity for up to 6 months.

**Once thawed**, the protein array glass slide (*Item A*) and Blocking Buffer (*Item D*) should be kept at -20 °C and all other components (*Items B, C, E, & F*) should be stored at 4 °C (check the table *below*).

**Please use kit within 6 months of purchase.**

**Please use kit within 3 months after reagents have been thawed.**

| <b>Item</b> | <b>Description</b>                      | <b>Storage</b>   |
|-------------|---|------------------|
| <b>A</b>    | Assembled Glass Slide                   | -20 °C           |
| <b>B</b>    | 1,000× Biotin-conjugated Anti-Human IgG | 4 °C             |
| <b>C</b>    | 1,500× HiLyte 555 Streptavidin          | 4 °C             |
| <b>D</b>    | Blocking Buffer                         | -20 °C           |
| <b>E</b>    | 20× Wash Buffer I                       | 4 °C             |
| <b>F</b>    | 20× Wash Buffer II                      | 4 °C             |
| <b>G</b>    | Adhesive Plastic Strips                 | Room Temperature |
| <b>H</b>    | 30 ml-Centrifuge Tube                   | Room Temperature |

## **3. Additional Materials Required**

- Distilled water
- Aluminum foil
- Small plastic boxes or containers
- Orbital shaker or oscillating rocker
- Pipettors, pipette tips and other common lab consumables
- Laser scanner for fluorescence detection

**RayBio® Analysis Tool** for Autoimmune Disease IgG Autoantibody Detection Array G1 (Cat. #. [PAH-AIDG-G1-SW](#)) is not provided in the kit. But this easy-to-use tool is very helpful for analyzing and organizing mass array data. It is recommended to order this analysis tool separately.

## II. Introduction

Autoantibodies are antibodies that react with self-antigens that are present in all human sera. Autoantibodies present in the sera of autoimmune disease (AID) patients play a central role in the diagnosis and classification of immune disorders. Therefore, autoantibodies detected before disease onset could serve as ideal biomarkers, enabling early and precise diagnosis of autoimmune diseases and targeting of therapeutic interventions.

The protein array is a high-throughput approach to autoantibody profiling. RayBio® Human Autoimmune Disease IgG Autoantibody Detection Array is a protein array which contains 33 major autoimmunogens, including dsDNA, presented in multiple autoimmune diseases in the target panel. The purified human autoimmunogen proteins are spotted onto a solid glass slide surface, and then probed for the presence of serum specific antibodies to these autoimmunogen targets. This detection kits can then be applied in high-throughput screening for specific targets based on IgG autoantibody levels present in test serum samples.

Custom protein arrays are also available from RayBiotech, Inc. You can select your own proteins of interest or provide your own target proteins to RayBiotech, Inc. After we receive your custom panel, we can produce tailored protein arrays for you.

### **1. Principle of the Assay**

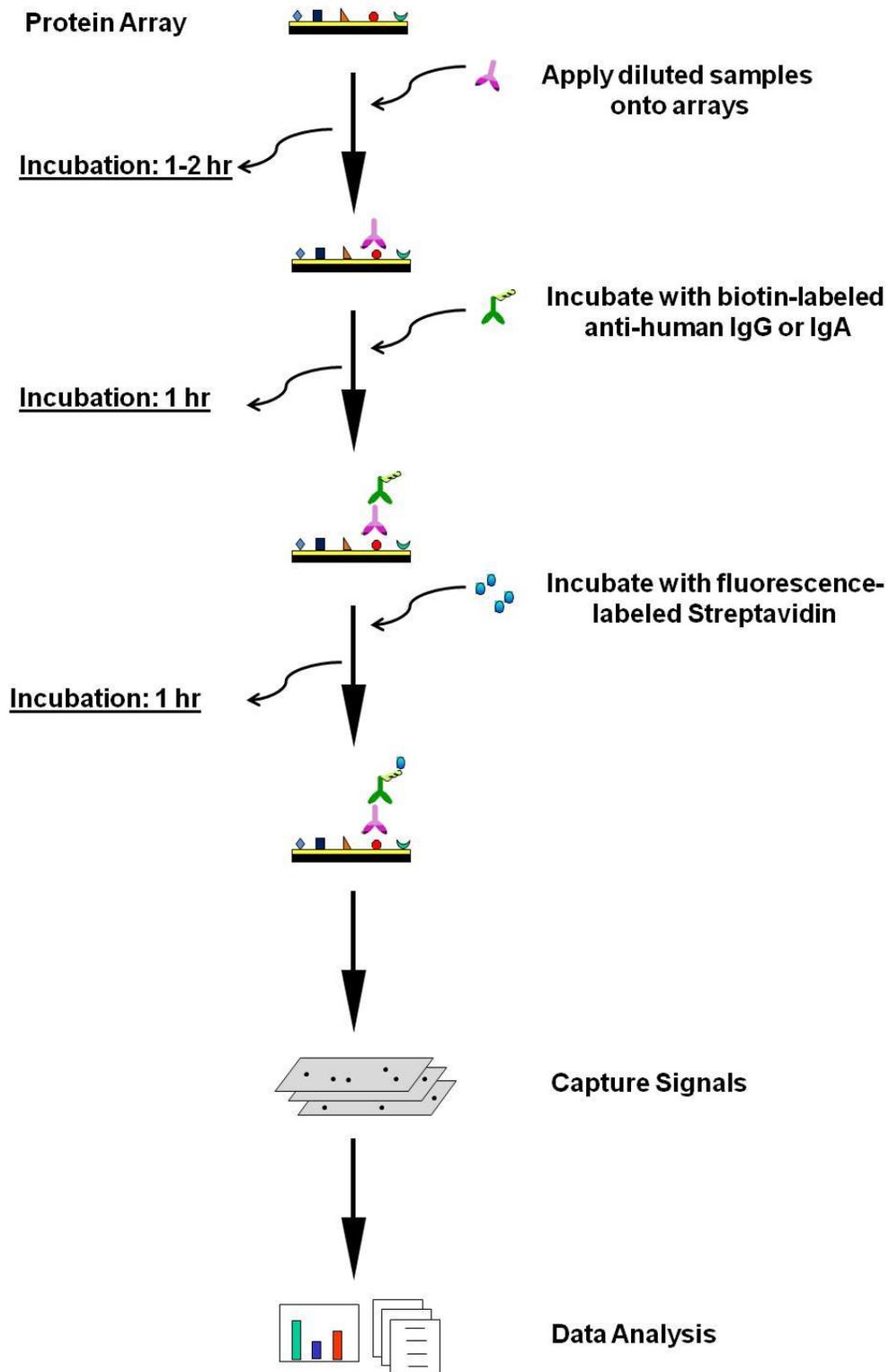
Microarray-based autoantibody detection is the foundation of high-throughput autoantibody screening in human samples. In this assay (see the flow chart below), the autoimmunogen proteins are arrayed in triplicate on a glass slide surface, and the assay includes multiple positive and negative controls to monitor each incubation step.

Serum or other fluid samples are then diluted and incubated on the protein arrays, allowing the serum IgG autoantibodies against these autoimmunogens to specifically bind. After comprehensive washing steps, the arrays are incubated with biotin-conjugated anti-human IgG, followed by addition of the fluorescence dye-conjugated streptavidin. The target fluorescence is then captured using a laser scanner and the presence of serum IgG specific to the arrayed targets can be evaluated.

## **2. Array Features**

- Low sample consumption: as little as 2  $\mu$ l of original serum sample required per array.
- Fully customizable: create a custom array from our list of targets.
- High sensitivity: both the biotin-streptavidin and the fluorescent detection enables the most sensitive assay available to measure serum IgG.
- High efficiency and accuracy: high-throughput screening of multiple targets in a single assay. Each slide can test up to 16 samples simultaneously, and contains internal positive controls to normalize between slides, thereby minimizing the variation from assay to assay. Additionally, the assay duration is less than 8 hours.
- Large dynamic range of detection (4 orders of magnitude) with highly accurate data that can be normalized between arrays.
- Affordable and simple to use.

## How Array Works:



### III. Overview and General Considerations

#### 1. Serum Sample Collection, Preparation, and Storage

- **Control samples:** It is suggested that normal human serum samples or a pooled sample derived from normal samples are included in the experiment as negative controls to define background signals.
- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid repeated freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- Avoid using hemolyzed serum or plasma as this may interfere with protein detection and/or cause a higher than normal background response.
- Always centrifuge the samples (5,000 g for 5 minutes at 4 °C) after thawing to remove any particulates that could interfere with detection.
- The conditions described in this manual are optimized already. If you experience high background, you may need to further dilute your samples and/or to wash slides in Wash Buffer I (*Item E*) overnight at 4 °C. If the signal is too weak, you may increase the volume of your samples and/or increase incubation times of one or more steps.

#### 2. Handling Glass Arrays

- The microarray slides are delicate. 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.
- 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 of the chamber assembly.



### 3. **Incubation**

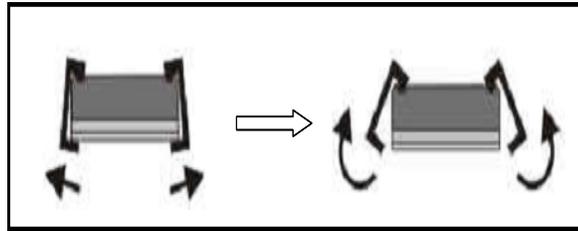
- Completely cover array area with sample or buffer during incubation steps.
- Cover the incubation chamber with adhesive strips (*Item G*) or plastic sheet protector during incubation to avoid drying, particularly when incubation lasts more than 2 hours or less than 70  $\mu$ l of sample or reagent is used.
- During incubation and wash steps, avoid foaming and remove any bubbles from the sub-array surface.
- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/second).
- 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 as shown in picture *above*.
- Several incubation steps such as blocking, sample incubation, biotin-conjugated antibody incubation, or fluorescence-conjugated streptavidin incubation may be done at 4 °C overnight. Before overnight incubations, cover the incubation chamber tightly (*Item G*) to prevent evaporation.
- Protect glass slides from direct, strong light and temperatures above room temperature.

### 4. **Layout of Glass Arrays**

- RayBio<sup>®</sup> Human Autoimmune Diseases IgG Autoantibody Detection Arrays are available as 16 sub-arrays per glass slide.
- Don't touch the printed surface of the glass slide. The 16-subarray glass slide has will have a tiny green mark or other marks to indicate in the bottom right corner of the printed side.

## 5. Incubation Chamber Disassembly

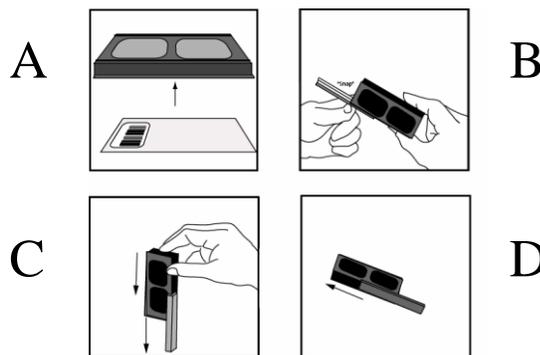
After completing the experiment, carefully disassemble the glass slide from the incubation frame and chamber by pushing clips outward from the sides, as shown *below*. Carefully remove the glass slide from the gasket. Don't touch the printed surface of the glass slide, which is on the same side as the barcode or other marks.



## 6. Incubation Chamber Assembly

After finishing your experiment, if you need to repeat any of the incubation or wash steps, you must first re-assemble the glass slides into the incubation chamber. Do this by following the steps as shown in the figures below. To avoid breaking the printed glass slide, it is recommended that you first practice assembling the device with a regular blank standard glass histology slide.

- 1) Apply slide to incubation chamber, barcode facing upward (A).
- 2) Gently snap one edge of a snap-on side (B).
- 3) Gently press other side against lab bench and push in lengthwise direction (C).
- 4) Repeat with the other side (D)



## IV. Protocol

The table below describes the steps and experimental outline required to perform the array detection using the Raybio® Autoimmune Disease IgG Autoantibody Detection Array kit. The whole procedure takes ~ 8 hours.

| Step | Action                                      | Time   |
|------|---|--------|
| 1    | Equilibrate kit to room temperature         | 30 min |
| 2    | Glass slide air-dry                         | 1 hour |
| 3    | Array blocking                              | 1 hour |
| 4    | Sample incubation                           | 1 hour |
| 5    | Array washing                               | 40 min |
| 6    | Biotin-conjugated anti-human IgG incubation | 1 hour |
| 7    | Array washing                               | 40 min |
| 8    | Cy3-conjugated streptavidin incubation      | 1 hour |
| 9    | Array washing and drying                    | 1 hour |

Before proceeding to the experiment, please refer to the following dilution chart to prepare samples/reagents for each step.

| Item | Description  | Dilution Fold | Diluent                  | Dilution Method  | Temporary Storage                | Shelf life                   |
|------|--|---------------|--------------------------|--|----------------------------------|------------------------------|
|      | Sample Serum   | 200           | Blocking Buffer (Item D) | Mix 1 µl serum with 199 µl of Blocking Buffer. Mix and spin down.                  | Fresh ice                        | Use immediately once diluted |
| B    | 1,000x Biotin-conjugated Anti-Human IgG, 1.5 µl/vial | 1,000         | Blocking Buffer (Item D) | Spin the vial first, then add 1.5 ml Blocking Buffer per vial. Mix and spin again. | Fresh ice                        | Use immediately once diluted |
| C    | 1,500x HiLyte 555 Streptavidin, 1 µl/vial            | 1,500         | Blocking Buffer (Item D) | Spin the vial first, then add 1.5 ml Blocking Buffer per vial. Mix and spin again. | Fresh ice. Protected from light. | Use immediately once diluted |
| E    | 20x Wash Buffer I                                    | 20            | Distilled water          | Dilute with 19-times distilled water. Mix well.                                    | Room temperature                 | 1 week                       |
| F    | 20x Wash Buffer II                                   | 20            | Distilled water          | Dilute with 19-times distilled water. Mix well.                                    | Room temperature                 | 1 week                       |

## 1. Blocking and Sample Incubation

- 1.1 Take the package containing the Assembled Glass Slide (*Item A*) from the freezer. Place **UNOPENED** package on the bench top for approx. 30 minutes, and allow the Assembled Glass Slide to equilibrate to room temperature.
- 1.2 Open package carefully and take the Assembled Glass Slide out of the sleeve (Do not disassemble the Glass Slide from the chamber assembly). Peel off the cover film and let Glass Slide Assembly air-dry in clean environment for 1 hour at room temperature.

**Note:** *Protect the slide from dust or other contaminants. Incomplete drying of slides before use may cause the formation of “comet tails”.*

- 1.3 Block sub-arrays by adding 100 µl of Blocking Buffer (*Item D*) into each well of Assembled Glass Slide (*Item A*) and incubating at room temperature for 1 hour. Ensure there are no bubbles on the array surfaces.

**Note:** *Be careful not to add reagents forcefully or directly to the glass slide. Always add reagents slowly to the sides of the plastic well assembly.*

- 1.4 Decant Blocking Buffer from each well completely.
- 1.5 If using serum samples, centrifuge the serum samples at 5,000 g for 5 minutes at 4 °C. Transfer the supernatants into new tubes.
- 1.6 Dilute serum samples 200-fold: mix 1 µl of serum with 199 µl of Blocking Buffer (*Item D*). Keep all diluted samples on ice.
- 1.7 Load 100 µl of diluted serum samples into each well. Remove any bubbles from the array surfaces. It is recommended to include control samples as well, for example, normal human serum samples.
- 1.8 Incubate arrays with gentle rocking or shaking at room temperature for 1 hour, or other conditions as appropriate.
- 1.9 Decant the samples from each well, and wash the wells 5 times with 150 µl of 1× Wash Buffer I (*Item E*). Wash at room temperature with gentle shaking for 5 minutes per wash. Completely remove 1× Wash Buffer I in each wash step.

**Note:** Dilute 20× Wash Buffer I (*Item E*) to 1× with distilled water. Avoid solution flowing into neighboring wells. If crystals have formed in the 20× concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved.

**1.10** Wash 2 times with 150 µl of 1× Wash Buffer II (*Item F*). Wash at room temperature with shaking for 5 minutes per wash. Completely remove 1× Wash Buffer II in each wash step. Incomplete removal of the wash buffer in each wash step may cause “dark spots” (Background signals higher than that of the spot).

**Note:** Dilute 20× Wash Buffer II (*Item F*) to 1× with distilled water. If crystals have formed in the 20× concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved.

## **2. Biotin-conjugated Anti-human IgG Incubation**

**2.1** Briefly spin down the vial of 1,000× Biotin-conjugated Anti-human IgG (*Item B*). Add 1.5 ml of Blocking Buffer (*Item D*) and mix well.

**2.2** Add 100 µl of 1,000-fold diluted Biotin-conjugated Anti-human IgG into each well.

**2.3** Incubate at room temperature for 1 hour.

**2.4** Wash with 1× Wash Buffer I as described in *Step 1.9*, then wash with 1× Wash Buffer II as described in *Step 1.10, above*.

## **3. Cy3-conjugated Streptavidin Incubation**

**3.1** Briefly spin the vial containing 1,500× Fluorescence-conjugated Streptavidin (*Item C*) prior to use. Add 1.5 ml of Blocking Buffer (*Item F*) and mix well.

**3.2** Add 100 µl of 1,500-fold diluted Fluorescence-conjugated Streptavidin into each well.

**3.3** Cover the incubation chamber with aluminum foil to avoid exposure to light or incubate in dark room.

**3.4** Incubate at room temperature for 1 hour with gentle rocking or shaking.

- 3.5 Wash with 1× Wash Buffer I as described in *Step 1.9*, then wash with 1× Wash Buffer II as described in *Step 1.10*, above.

#### 4. **Fluorescence Detection**

- 4.1 Decant excess 1× Wash Buffer II from wells.
- 4.2 Carefully disassemble the glass slide from the incubation frame and chamber (see details in Section III).

*Note: Be careful not to touch the printed surface of the glass slide, which is on the same side as the barcode or other marks.*

- 4.3 Place the whole slide in the included 30-ml Centrifuge Tube (*Item H*) or a glass slide holder with the cap. Add enough 1× Wash Buffer I (about 30 ml) to cover the whole slide and gently shake or rock at room temperature for 15 minutes. Decant 1× Wash Buffer I.
- 4.4 Wash with 1× Wash Buffer II (about 30 ml) with gentle shaking at room temperature for 10 minutes. Decant 1x Wash Buffer II.
- 4.5 Take glass slide out of the wash container, and gently apply suction with a pipette to remove any water droplets. Do not touch the array area, only the unprinted area. Let slide air-dry completely at least 20 minutes (protect from light).

*Note: Make sure the slides are **absolutely** dry before starting the scanning procedure or storage. High background can result from incomplete drying of the slide*

- 4.6 Slide scanning: the array signals can be visualized through use of a laser scanner equipped with a Cy3/green channel capable wavelength, such as an Axon GenePix. Scan all slides at the same PMT.

For additional scanners and slide scanning information, please visit our website:  
[www.raybiotech.com/Tech-Support/Laser\\_Scanners\\_for\\_Glass\\_Slide\\_Arrays.pdf](http://www.raybiotech.com/Tech-Support/Laser_Scanners_for_Glass_Slide_Arrays.pdf)

**Notes:**

- *It is possible the signal intensity for different targets will vary greatly in the same array; thus, we recommend performing multiple scans, starting with a low PMT for the high signals, then a higher PMT for low signals.*
- *Although we recommend scanning slides right after the experiment, you can also store the slide at room temperature or -20 °C in dark for several days. The Cy3 fluor dye used in this kit is very stable at room temperature and resistant to photo bleaching on completed glass slides.*

Don't have a laser scanner? Just send your slides to us and we can scan them for you. For details, please visit "**Array Scanning and Analysis Service**" at our website: [www.raybiotech.com/array-scanning-and-analysis-service.html](http://www.raybiotech.com/array-scanning-and-analysis-service.html)

## V. Data Analysis

### 1. Data Extraction

The captured array signal can be extracted with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, etc.) associated with the laser scanner. Tips in data extraction:

- Ignore any comet tails.
- Define the area for signal capture for all spots, usually 100-120 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.

### 2. Control Systems

To help data analysis, multiple following positive controls and negative controls are included on arrays for data normalization, array orientation determination, background evaluation, etc. Beside the normal serum controls, the comprehensive internal controls help to monitor the major assay steps, data normalization, background reading, etc. The following table describes all array controls and their functions.

| Controls          |   | Roles  |
|-------------------|---|--|
| Positive Controls | Biotin-BSA<br>(bovine serum albumin)                            | Array orientation  |
|                   |   | Data normalization   |
|                   |   | Evaluate the activity of fluorescence-dye labeled streptavidin |
| Human IgG         | Evaluate the activity of biotin-labeled anti-human IgG antibody |  |
| Negative Controls | Water   | Evaluate the background level                                  |
|                   | 0.1% BSA-PBS  | Evaluate the background level                                  |

### 3. Data Normalization

The raw data normalization is used to compare data between arrays (i.e., different samples) by accounting for the differences in signal intensities of **the positive**

**control spots** (for example, the printed biotin-BSA or IgG controls) on those arrays. The positive control is a controlled amount of biotinylated protein printed on the arrays in triplicate. The amount of signal from each of those spots is dependent on the amount of the reporter (Cy3-streptavidin) bound to biotinylated protein.

Since the reporter amount proportionally affects the signal intensity of every spot on the array, the differences in the signal of positive controls (biotin-BSA) between arrays will accurately reflect the differences between other spots on those arrays.

To normalize the data, one array must be defined as the “**Reference Array**” to which the signals of other “**Sample Arrays (s)**” are normalized. It is up to the customers to define which array should be the reference. In our analysis tool (Cat #. **PAH-AIDG-G1-SW**), Sample 1 will automatically be assigned as the reference array and the signal intensities of other subarrays will be calculated according to the following formula:

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

- P1 = the average signal density of positive control spots on the reference array
- P(y) = the average signal density of positive control spots on array y
- X(y) = the signal density for a particular spot on Array for sample “y”
- X(Ny)= the normalized value for that particular spot "X" on Array for sample "y"

#### **4. Threshold of significant difference in expression**

After subtracting background signals and normalizing to positive controls (biotin-BSA), the comparison of signal intensities for each target between and among array images can be used to determine relative differences in expression levels of each analyte between samples or groups.

Any  $\geq 10$ -fold increase in signal intensity for a single analyte between samples (for example, the signal ratio of normal samples and patient’s samples) or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background.

## 5. RayBio® Analysis Tool

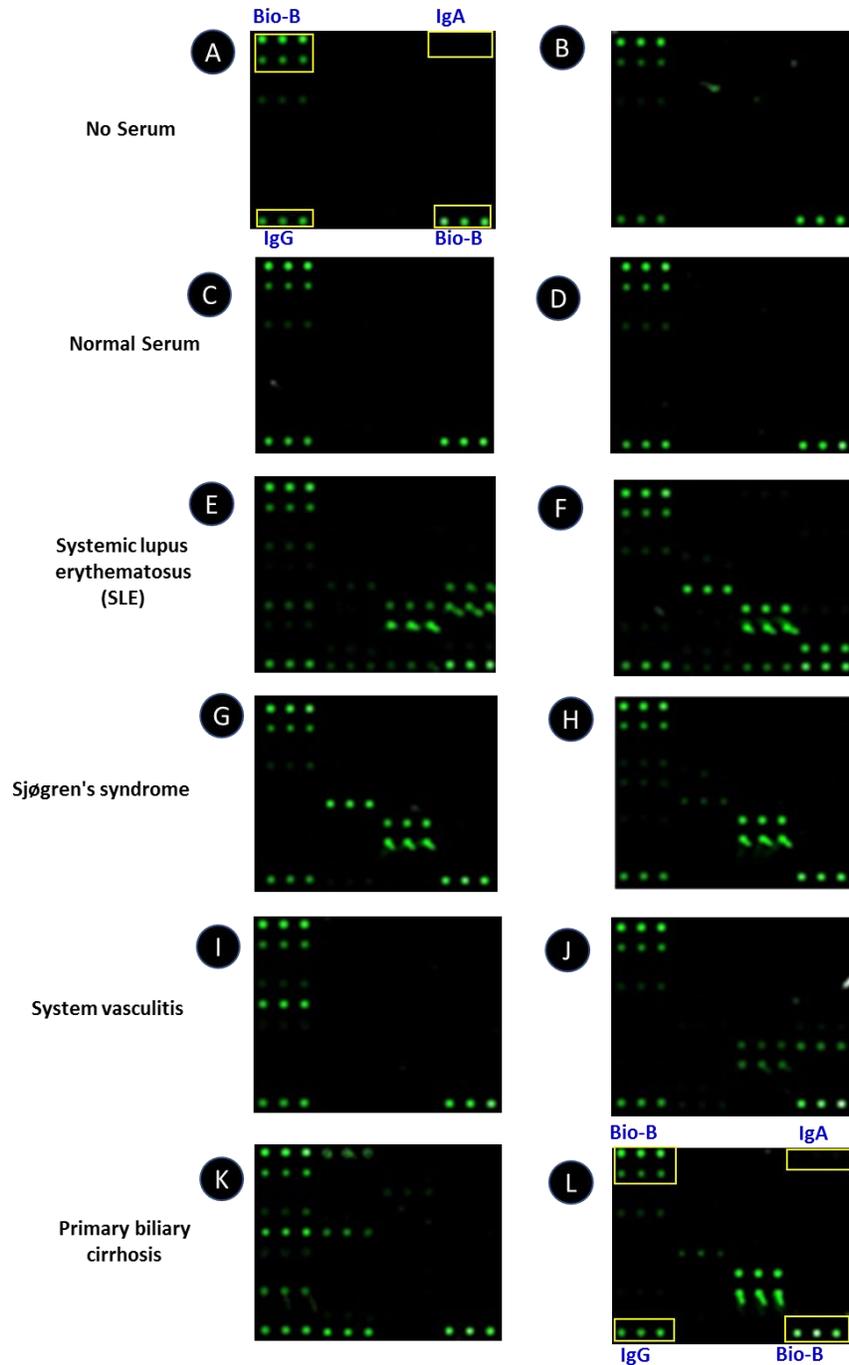
The signal intensities obtained from laser scanner can simply be imported into our analysis tool of Autoimmune Diseases IgG Autoantibody Detection Array (Cat. #. **PAH-AIDG-G1-SW**). This analysis tool is very simple and affordable, which will not only assist in compiling and organizing your data, but also reduces your calculations to a “copy and paste” step. The analysis tool will help you:

- Locate your signal intensities to array map
- Protein list sorting
- Average signal intensities
- Subtract background
- Normalize the data from different samples
- Obtain protein level comparison charts among different samples

## 6. Interpretation of Results

The following Figure 1 represents a typical result using serum on the RayBio® Autoimmune Disease IgG Autoantibody Detection Array (Cat #. PAH-AIDG-G1). The protein arrays were incubated with 200-fold diluted serum samples from normal individuals and patients with different autoimmune diseases. After washing steps, the arrays were sequentially incubated with biotin-labeled anti-human IgG antibody and fluorescent dye-labeled streptavidin. The signals from the bound autoantibodies were captured with a fluorescence scanner.

Sample diluent alone (Figs. 1A & 1B) and normal sera (Figs. 1C & 1D) were used as negative controls, and the arrays signals indicate that no significant background occurred. In some cases, a negligible signal can be observed, which may be caused by non-specific binding derived from the biotin-labeled anti-human IgG antibody and/or fluorescent dye-labeled streptavidin. However, these weak background signals will be subtracted in subsequent data analysis procedures and will not affect sample test results. A strong binding signal was detected in multiple targets among different autoimmune disease patients: systemic lupus erythematosus (Figs.1E & 1F), Sjögren's syndrome (Figs.1G & 1H), system vasculitis (Figs.1I & 1J), and primary biliary cirrhosis (Figs.1K & 1L).



**Fig 1. Typical result using serum on the RayBio® Autoimmune Disease IgG Autoantibody Detection Array.** The protein arrays were incubated with diluent alone (A, B), diluted serum samples derived from normal individuals (C, D) and those from various autoimmune disease patients (E-L). Yellow boxes, show various controls as indicated (biotin-labeled BSA, human IgG and IgA).

## VI. Appendix

### 1. Array Map

RayBio® Human Autoimmune Disease IgG Autoantibody Detection Array G1 is printed in a 10 spot × 12 spot format (see table *below*). 33 autoimmunogens are printed in triplicate on the array along with a series of positive controls (Biotin-BSA, human IgG) and negative controls (water, 0.1% BSA in PBS, human IgA).

For your convenience, or any editing, please visit our website <http://www.raybiotech.com/> to download this array map in the Excel format.

|    |                     |   |   |                  |   |   |                       |   |   |                         |    |    |    |
|----|---------------------|---|---|------------------|---|---|-----------------------|---|---|-------------------------|----|----|----|
|    | 1                   | 2 | 3 | 4                | 5 | 6 | 7                     | 8 | 9 | 10                      | 11 | 12 |    |
| 1  | POS, Biotin-BSA (1) |   |   | #8, gp-210       |   |   | #18, Mi-2             |   |   | Human IgA               |    |    | 1  |
| 2  | POS, Biotin-BSA (2) |   |   | #9, Histone H2A  |   |   | #19, MPO              |   |   | NEG, Blank              |    |    | 2  |
| 3  | #1, PL12            |   |   | #10, Histone H2B |   |   | #20, Nup62            |   |   | 0.1% BSA-PBS            |    |    | 3  |
| 4  | #2, KRT8            |   |   | #11, KRT19       |   |   | #21, CCP peptide      |   |   | #28, sp100              |    |    | 4  |
| 5  | #3, CENP-B          |   |   | #12, Jo-1        |   |   | #22, PCNA             |   |   | #29, PL-7               |    |    | 5  |
| 6  | #4, CENP-A          |   |   | #13, La/SS-B     |   |   | #23, Proteinase-3     |   |   | #30, Thyroglobulin (TG) |    |    | 6  |
| 7  | #5, dsDNA           |   |   | #14, LC-1        |   |   | #24, Ro/SS-A (52 kDa) |   |   | #31, TPO                |    |    | 7  |
| 8  | #6, Gliadin         |   |   | #15, KRT20       |   |   | #25, Ro/SS-A (60 kDa) |   |   | #32, tTG                |    |    | 8  |
| 9  | #7, EJ              |   |   | #16, M/Scl 100   |   |   | #26, Scl-70           |   |   | #33, U1-snRNP70 kDa     |    |    | 9  |
| 10 | Human IgG           |   |   | #17, M2          |   |   | #27, Sm/Smith/snRNP   |   |   | POS, Biotin-BSA (1)     |    |    | 10 |
|    | 1                   | 2 | 3 | 4                | 5 | 6 | 7                     | 8 | 9 | 10                      | 11 | 12 |    |

### 2. Array List

The RayBio® Human Autoimmune Disease IgG Autoantibody Detection Array G1 includes 33 essential autoimmunogens discovered in autoimmune diseases patients (see list, *next page*). If you want to add your autoimmunogens of interest or remove any targets from this list, please contact us so we can customize your specific array as needed.

For your convenience, or any editing, this autoimmunogen target list in the Excel format can be downloaded at our website <http://www.raybiotech.com/>.

RayBio® Human Autoimmune Disease IgG Autoantibody  
Detection Array G1

## Target List

| No. | Autoimmunogen                         | No. | Autoimmunogen                   |
|-----|---------------------------------------|-----|---------------------------------|
| #1  | Alanyl-tRNA Synthetase (PL-12)        | #18 | Mi-2                            |
| #2  | KRT8                                  | #19 | Myeloperoxidase (MPO), p-ANCA   |
| #3  | Centromere B (CENP-B)                 | #20 | Nup62                           |
| #4  | Centromere Protein A (CENP-A)         | #21 | CCP peptide                     |
| #5  | dsDNA                                 | #22 | PCNA                            |
| #6  | Gliadin                               | #23 | Proteinase-3 (PR3), c-ANCA      |
| #7  | Glycyl-tRNA Synthetase (EJ)           | #24 | Ro/SS-A (52 kDa)                |
| #8  | gp-210                                | #25 | Ro/SS-A (60 kDa)                |
| #9  | Histone H2A                           | #26 | Scl-70 (DNA topoisomerase-1)    |
| #10 | Histone H2B                           | #27 | Sm/Smith/snRNP core protein     |
| #11 | KRT19                                 | #28 | sp100                           |
| #12 | Jo-1 (histidyl tRNA synthetase)       | #29 | Threonyl-tRNA synthetase (PL-7) |
| #13 | La/SS-B                               | #30 | Thyroglobulin (TG)              |
| #14 | Liver cytosolic antigen type 1 (LC-1) | #31 | Thyroid peroxidase (TPO)        |
| #15 | KRT20                                 | #32 | Tissue Transglutaminase (tTG)   |
| #16 | M/Scl 100                             | #33 | U1-snRNP70 kDa                  |
| #17 | M2                                    | #33 | U1-snRNP70 kDa                  |

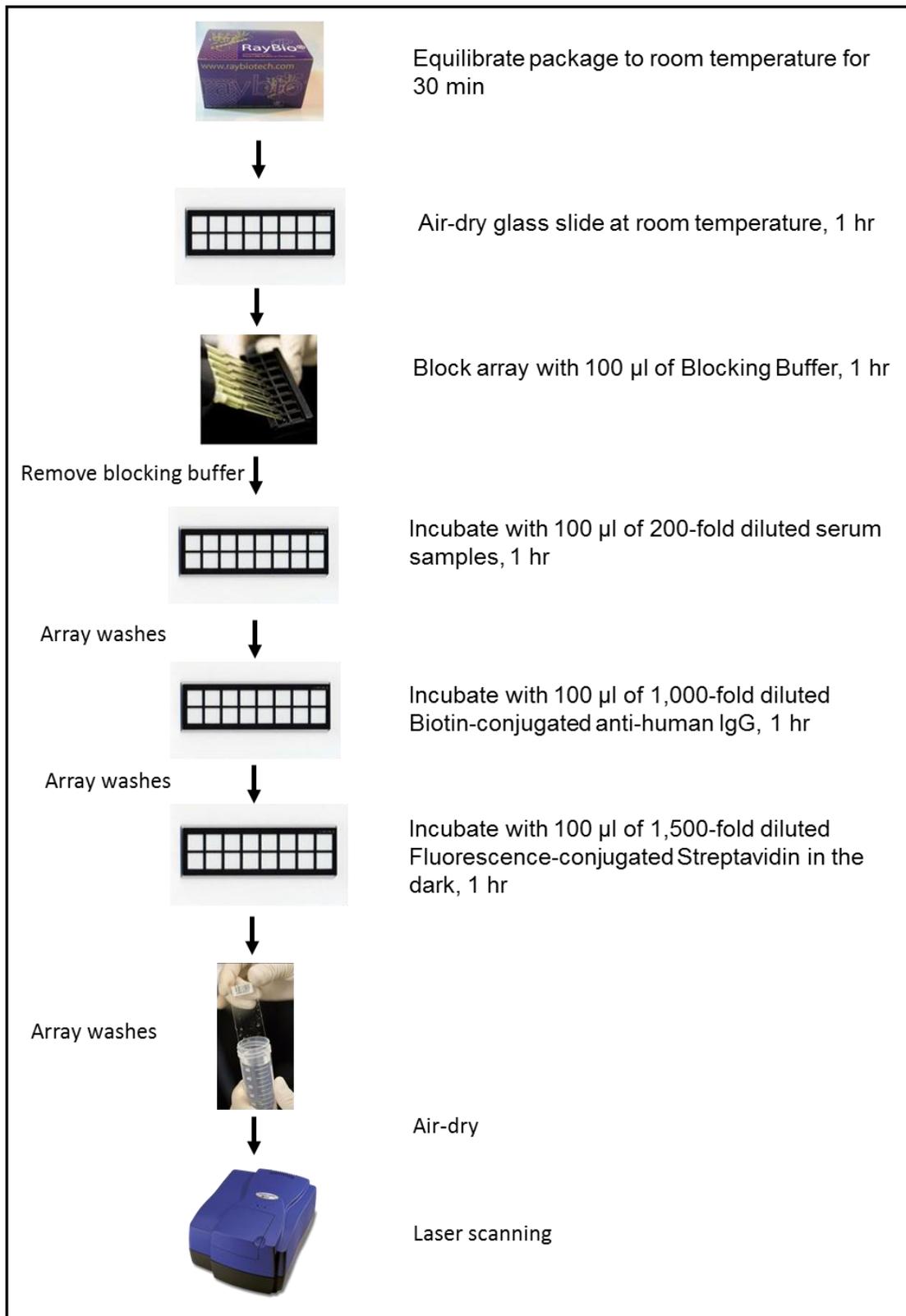
### 3. Troubleshooting Guide

| <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 times                                   | Ensure sufficient incubation time or change sample incubation to an overnight step |
|                        | Protein or antibody concentrations in sample are too low | Dilute starting sample less or concentrate sample                                  |
|                        | Improper storage of kit                                  | Store kit as suggested temperature; Don't freeze/thaw the slide                    |
| <b>High Background</b> | Excess of protein or antibody                            | Further dilute protein or antibody   |
|                        | Excess of streptavidin                                   | Further dilute streptavidin  |
|                        | Overexposure   | Lower the laser power  |
|                        | 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    |
|                        | Dark Spots   | Completely remove wash buffer in each wash step                                    |
|                        | Insufficient wash  | Increase wash time and use more wash buffer  |
| <b>Uneven Signal</b>   | Bubbles formed during incubation                         | Handle and pipette solutions more gently; De-gas solutions prior to use            |
|                        | Reagent evaporation                                      | Cover the incubation chamber with adhesive film during incubation                  |
|                        | Arrays are not completely covered by reagent             | Prepare more reagent and completely cover arrays with solution                     |

#### **4. Reference List**

- Casali P, Schettino EW. Structure and function of natural antibodies. *Curr Top Microbiol Immunol.* 1996; 210:167–179
- Arbuckle MR, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med.* 2003; 349:1526–1533
- Mitchison HC, et al. Symptom development and prognosis in primary biliary cirrhosis: a study in two centers. *Gastroenterology.* 1990; 99:778–784
- Rapoport B, McLachlan SM. Thyroid autoimmunity. *J Clin Invest.* 2001;108:1253–1259
- William H. Robinson, et al. Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nature Medicine.* 2002; 8, 295 - 301
- Hueber W, Robinson WH. Proteomic biomarkers for autoimmune disease. *Proteomics.* 2006; 6(14):4100-5
- Stephen Lam, Peter Boyle, Graham F. Healey. EarlyCDT-Lung: An Immunobiomarker Test as an Aid to Early Detection of Lung Cancer. *Cancer Prev Res.* 2011; 4(7): 1126-1134
- Petra Leidinger, Andreas Keller, Nicole Ludwig. Toward an early diagnosis of lung cancer: An autoantibody signature for squamous cell lung carcinoma. *Int. J. Cancer.* 2008; 123, 1631–1636
- Chen, G., Wang, X., Yu, J., et al. Auto-antibody profiles reveal ubiquitin 1 as a humoral immune response target in lung adenocarcinoma. *Cancer Res.* 2007; 67, 3461-3467
- Huang, R.P. Cytokine antibody arrays: a promising tool to identify molecular targets for drug discovery. *Comb. Chem. High Throughput. Screen.* 2003a; 6, 769-775
- Huang, R.P. Protein arrays, an excellent tool in biomedical research. *Front Biosci.* 2003b; 8, D559-D576

## 5. Quick Protocol



## 6. Experiment Record Form

- Date \_\_\_\_\_
- File Name \_\_\_\_\_
- Laser Scanner \_\_\_\_\_
- Laser Power \_\_\_\_\_
- PMT \_\_\_\_\_

Slide # \_\_\_\_\_

| Well No. | Sample Name | Dilution Factor |
|----------|-------------|-----------------|
| 1        |             |                 |
| 2        |             |                 |
| 3        |             |                 |
| 4        |             |                 |
| 5        |             |                 |
| 6        |             |                 |
| 7        |             |                 |
| 8        |             |                 |
| 9        |             |                 |
| 10       |             |                 |
| 11       |             |                 |
| 12       |             |                 |
| 13       |             |                 |
| 14       |             |                 |
| 15       |             |                 |
| 16       |             |                 |

Slide # \_\_\_\_\_

| Well No. | Sample Name | Dilution Factor |
|----------|-------------|-----------------|
| 1        |             |                 |
| 2        |             |                 |
| 3        |             |                 |
| 4        |             |                 |
| 5        |             |                 |
| 6        |             |                 |
| 7        |             |                 |
| 8        |             |                 |
| 9        |             |                 |
| 10       |             |                 |
| 11       |             |                 |
| 12       |             |                 |
| 13       |             |                 |
| 14       |             |                 |
| 15       |             |                 |
| 16       |             |                 |

**Note:**

RayBio® is the trademark of RayBiotech, Inc.

This product is intended for research purposes only and is not to be used for clinical diagnostics. 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 these materials.

Products are guaranteed for six 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.

This product is for research use only.



©2011 RayBiotech, Inc.