

RayBio® Human Lung Cancer IgG Autoantibody Detection Array G1

Glass–chip-based protein arrays

User Manual

(Revised 06.04.2020. Ver 2)

**For simultaneous detection of
30 human autoantibodies (IgG type)
from lung cancer patients' sera or fluids**

**Human Lung Cancer IgG Autoantibody Detection Array G1
(Cat #. PAH-LCAG-G1)**

**Human Lung Cancer IgG Autoantibody Detection Array G1 Service
(Cat #. PAH-LCAG-G1-SERV)**

Please read manual carefully before starting experiment

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



RayBiotech, Inc.

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

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Array Overview

Autoimmunogen Detected	30 targets
Array Format	Glass slide
Autoantibody Type Detected	Human IgG
Array Size	8 and 16 sample detection per glass slide
Detection Method	Fluorescence (Cy3 equivalent dye) with laser scanner
Sample Volume	100 µL diluted sample per array
Assay Duration	< 8 hours

Additional Custom Protein Array Services We Provide:

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

1. Experiment Design

RayBiotech's protein array experts can assist you in your experiment 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.

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I. Kit Contents and Storage

This manual provides the guidelines and instructions for detecting IgG type autoantibody from lung cancer patient's serum or other fluids using RayBio® Human Lung Cancer IgG Autoantibody Detection Array Kit.

1. Array Kit Components

Each array kit contains the following components, depending on the sample kit sizes (8 and 16 sample kits).

Item	Description	Cat #. PAH-LCAG-G1-8	Cat #. PAH-LCAG-G1-16
A	RayBio® Human Lung Cancer IgG Auto-antibody Detection Array, Assembled Glass Slide	1 slide (8 sub-arrays)	1 slide (16 sub-arrays)
B	1,000× Biotin-conjugated Anti-Human IgG 1.5 µl/vial	1 vial	2 vials
C	1,500× Cy3 equivalent dye-conjugated Streptavidin 1 µl/vial	1 vial	2 vials
D	Blocking Buffer	1 bottle	1 bottle
E	20× Wash Buffer I	1 bottle	1 bottle
F	20× Wash Buffer II	1 bottle	1 bottle
G	Adhesive Plastic Strips	1 strip	1 strip
H	30 ml-Centrifuge Tube	1 tube	1 tube
I	User Manual	Please download from http://www.raybiotech.com/	
J	Array Target List		
K	Array Map Template		

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.

Item	Description	Storage
A	Assembled Glass Slide	-20 °C
B	1,000× Biotin-conjugated Anti-Human IgG	4 °C
C	1,500× Cy3 equivalent dye-conjugated Streptavidin	4 °C
D	Blocking Buffer	-20 °C
E	20× Wash Buffer I	4 °C
F	20× Wash Buffer II	4 °C
G	Adhesive Plastic Strips	Room Temperature
H	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 Lung Cancer IgG Autoantibody Detection Array G1 (Cat. #. PAH-LCAG-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

Lung cancer is the worldwide leading cause of cancer related mortality. Patients with non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) can mount a humoral immune response to their cancers. It is well established that cancer patients produce autoantibodies to tumor proteins that are mutated, over-expressed, misfolded, ectopically presented, anomalously glycosylated, or aberrantly degraded, etc.

Autoantibodies have been found in the blood of patients who develop lung cancer up to 5 years before tumors can be detected by spiral computed tomography scans. Therefore, monitoring people at increased risk of lung cancer for the presence of serum autoantibodies may allow earlier diagnosis of the disease and earlier therapeutic intervention.

RayBio® Human Lung Cancer IgG Autoantibody Detection Arrays are a series of protein array developed by RayBiotech, Inc., and 30 major autoimmunogens in lung cancer are selected in the target panel. The purified recombinant human autoimmunogens 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 specific targets based on IgG autoantibody levels present in patient 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 produce this tailored protein arrays for you.

1. Principle of the Assay

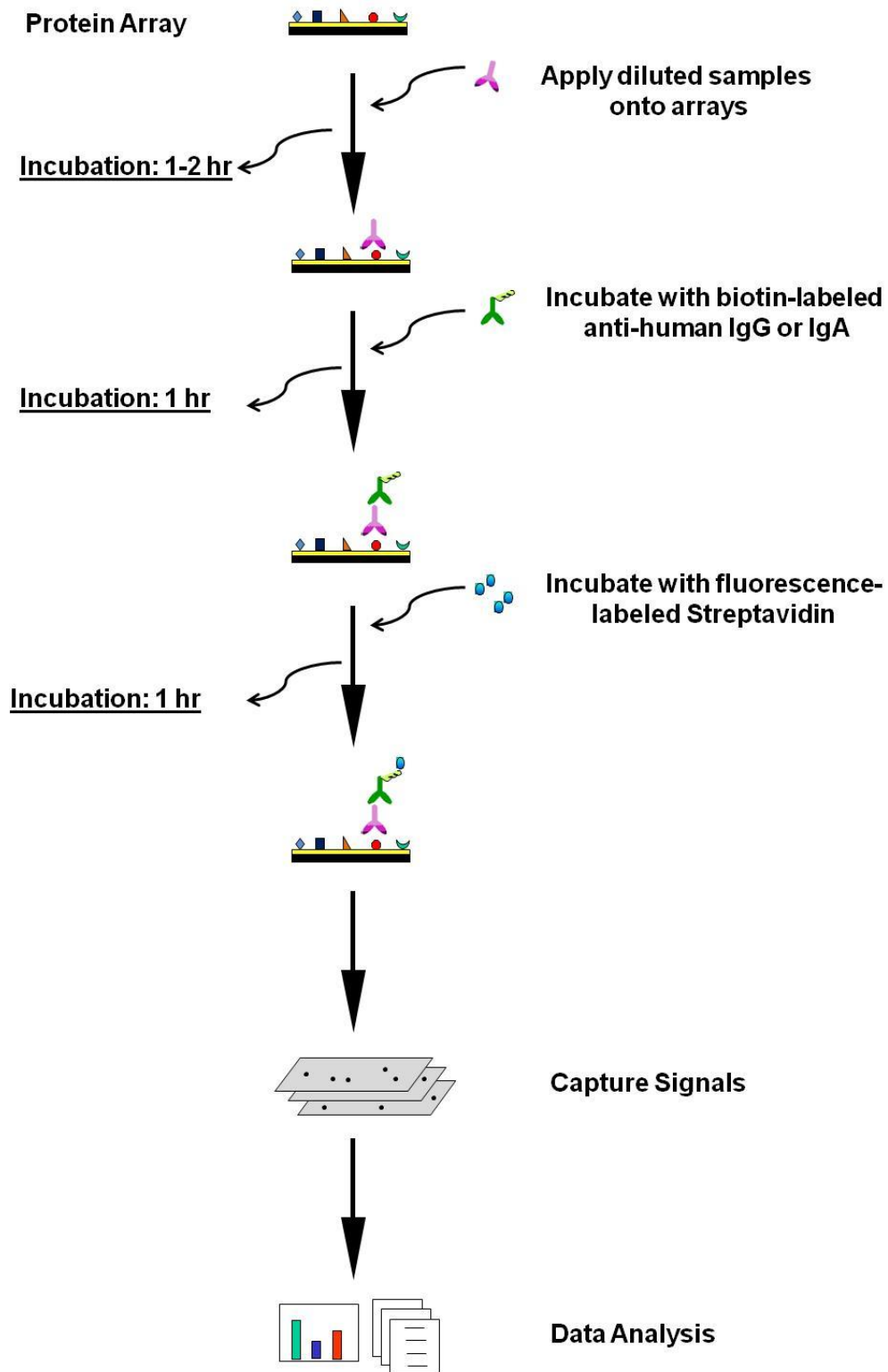
Microarray-based autoantibody detection is the foundation of high throughput autoantibody screening in human samples. In this assay (see the flow chart on the *next page*), the autoimmunogen proteins are arrayed in triplicate on a glass slide surface, the assay includes multiple positive and negative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

Serum or other fluid samples are diluted and incubated on the protein arrays, allowing 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 μ l of original serum sample required per array.
- Fully customizable: create a custom array from our list of targets.
- High sensitivity: both biotin-streptavidin pair and fluorescent detection enable 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 combined serum pool derived from normal samples are included in the array tests 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 in order to remove any particulates that could interfere with detection.
- The condition described in this manual is 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.

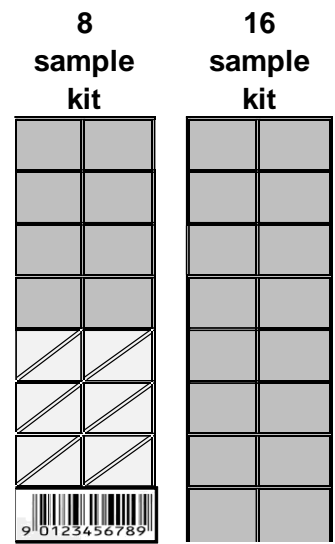
3. Incubation

- 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 μ 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

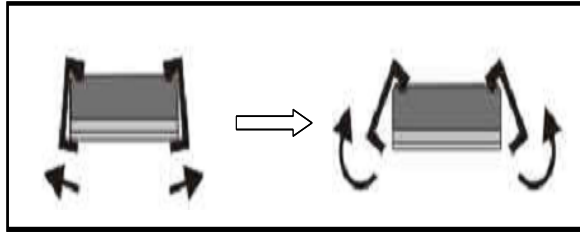
- RayBio® Human Lung Cancer IgG Autoantibody Detection Arrays are available in 8 and 16 sub-arrays per glass slide (see *picture on right*). Only the sub-arrays printed with autoimmunogen proteins should be loaded with reagents or samples, as the other wells are **blank**.



- Don't touch the printed surface of the glass slide, which is on the same side as the barcode. 16-subarray glass slide has no place to print bar code and normally a tiny green mark is indicated in the bottom right corner of the printed side.

5. Incubation Chamber Disassembly

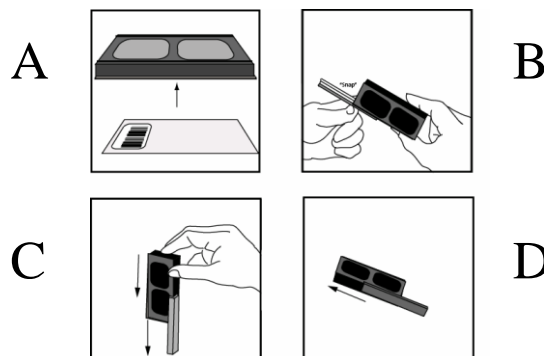
Carefully disassemble the glass slide from the incubation frame and chamber by pushing clips outward from the sides, as shown *below*. Carefully remove the glass slide from the gasket. Don't touch the printed surface of the glass slide, which is on the same side as the barcode or other marks.



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 Raybio® Lung Cancer 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 following dilution chart to prepare samples/reagents for each step.

Item	Description	Dilution Fold	Diluent	Dilution Method	Temporary Storage	Shelf life
	Patient 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	1,000	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 Cy3 equivalent dye-conjugated Streptavidin	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 others contaminants. Incomplete drying of slides before use may cause the formation of “comet tails”.*

- 1.3 Block sub-arrays by adding 100 μ l 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: *Only add reagents to wells printed with autoimmunogen proteins. 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 μ l of serum with 199 μ l of Blocking Buffer (*Item D*). Keep all samples on ice.
- 1.7 Load 100 μ l 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 condition as appropriate.
- 1.9 Decant the samples from each well, and wash the wells 5 times with 150 μ l of 1 \times Wash Buffer I (*Item E*). Wash at room temperature with gentle shaking for 5 minutes per wash. Completely remove 1 \times 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 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 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 Equivalent Dye-conjugated Streptavidin Incubation

- 3.1** Brief spin the vial containing 1,500× Fluorescence-conjugated Streptavidin (*Item C*) prior to use. Add 1.5 ml of Blocking Buffer (*Item D*) 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 web:

www.raybiotech.com/Tech-Support/Laser_Scanners_for_Glass_Slide_Arrays.pdf

Notes:

- *In case the signal intensity for different targets varies greatly in the same array, we recommend performing multiple scans, starting with a low PMT for high signal, then a higher PMT for low signal.*
- *Although we recommend scanning slides right after the experiment, you also can 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

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. Following table describes all array controls and their functions.

Controls		Roles
Positive Controls	Biotin-BSA (bovine serum albumin)	Array orientation
		Data normalization
		Evaluate the activity of fluorescence-dye labeled streptavidin
	Human IgG (serial diluted)	Array orientation
		Data normalization
		Evaluate the activity of biotin-labeled anti-human IgG antibody
Negative Controls	PBS	Evaluate the background level
	0.1% BSA-PBS	Evaluate the background level
	Normal Human Serum	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** (*r*)” 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. The normalized values are calculated as follows:

$$nX_s = X_s \times \frac{P_r}{P_s}$$

- ***P_r***: the average signal value of all biotin-BSA spots on the reference array (*r*)
- ***P_s***: the average signal value of all biotin-BSA spots on the sample array (*s*)
- ***X_s***: the signal value for a particular spot (*X*) on sample array (*s*)
- ***nX_s***: the normalized *X_s* value

4. Threshold of significant difference in expression

After subtracting background signals, normalization to positive controls (biotin-BSA), and removing the signals from normal human serum samples, 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 ≥ 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.

5. RayBio® Analysis Tool

The signal intensities obtained from laser scanner can simply be imported into our analysis tool of Lung Cancer IgG Autoantibody Detection Array (Cat. #. PAH-LCAG-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 serum test result of RayBio® Lung Cancer Autoantibody Detection Array. The protein arrays were incubated with 1:200 diluted serum samples. After washing steps, the arrays were sequentially incubated with biotin-labeled anti-human IgG antibody and fluorescent dye-labeled streptavidin. The bound autoantibody signals on arrays were captured by fluorescence scanner. Fig.1A shows there is no any background observed derived from biotin-labeled anti-human IgG antibody and/or fluorescent dye-labeled streptavidin. Fig.1B shows the result of one normal human serum. Slight binding activity in some targets was observed. This background will be subtracted in data analysis procedures without affecting sample data analysis. Fig.1C and Fig.1D were the results from two individual lung cancer patients and apparently several autoantibodies have been detected.

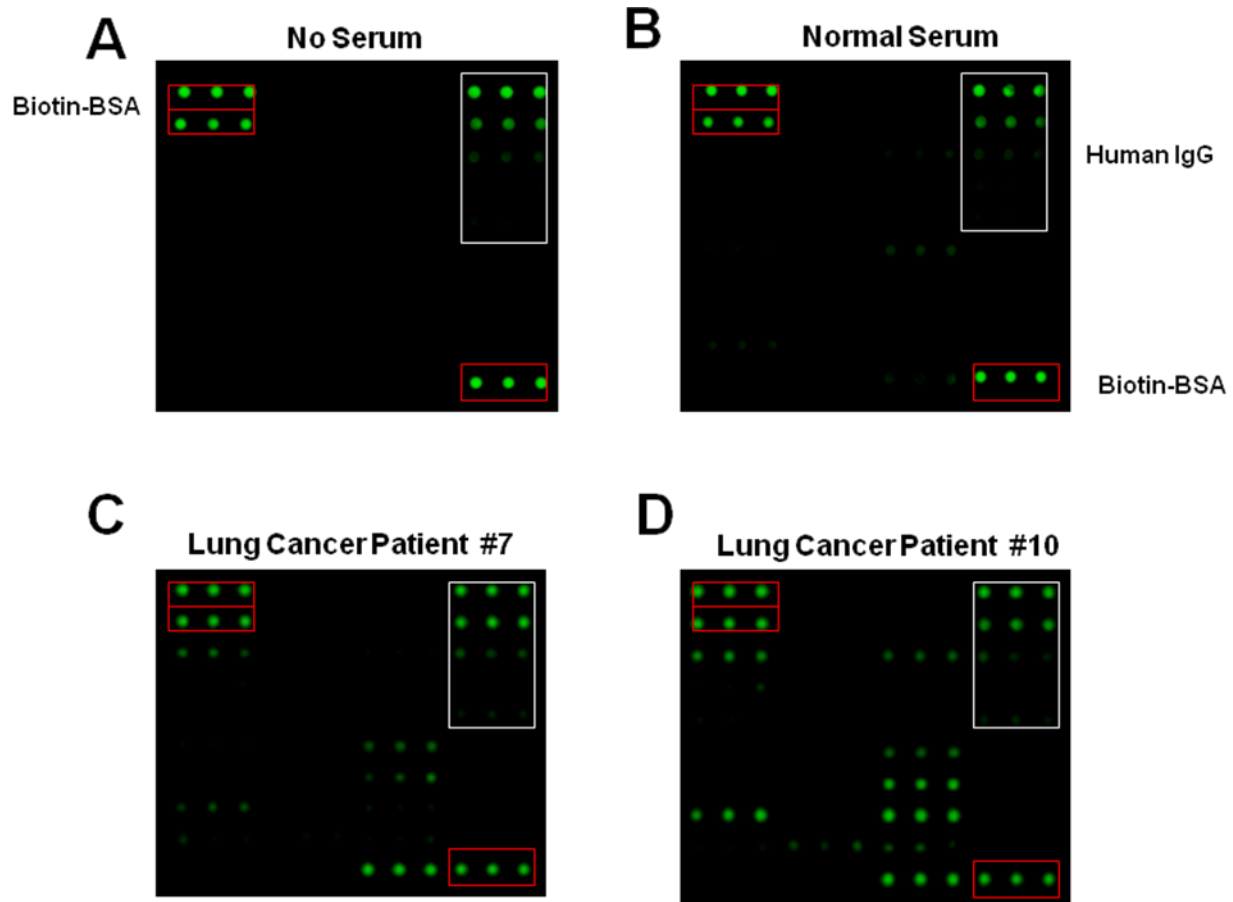


Fig 1. Human serum test of RayBio® Lung Cancer Autoantibody Detection Array. The protein arrays were respectively incubated with diluted serum samples derived from normal human (B), lung cancer patient #7 (C), and lung cancer patient #10 (D), or without human serum to view the background (A). Red boxes, biotin labeled BSA positive controls. White boxes, human IgG positive controls.

VI. Appendix

1. Array Map

RayBio® Human Lung Cancer IgG Autoantibody Detection Array G1 is printed in 10 spot x 12 spot format (see table *below*). 30 autoimmunogens are printed in triplicate, including a series of positive and negative controls.

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	Biotin-BSA (1)			Cyclin B1			LAMR1			Human IgG (1)		
2	Biotin-BSA (2)			Cyclin D1			MAGEA4			Human IgG (2)		
3	14-3-3 theta			Dkk-1			MALAT1			Human IgG (3)		
4	Annexin A1			GBU4-5			NY-ESO-1			Human IgG (4)		
5	Annexin A2			HSP70-9B/HSPA9/GRP75			p53			Human IgG (5)		
6	CAGE			Hu-D			Paxillin			Survivin		
7	Cathepsin D			IMDH2			PGAM1			Ubiquilin-1		
8	CDK2			IMP1			PGP9.5			0.1% BSA-PBS		
9	c-myc			IMP2/IGF2BP2/p62			Recoverin			PBS		
10	Cyclin A			IMP3			SOX2			Biotin-BSA (1)		

2. Array List

RayBio® Human Lung Cancer IgG Autoantibody Detection Array G1 includes 30 essential autoimmunogens discovered in lung cancer patients (see table, *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 Lung Cancer IgG Autoantibody
Detection Array G1

Target List

No.	Auto-immunogen	Gene Symbol	UniProt Accession #
#1	14-3-3 theta	YWHAQ	P27348
#2	Annexin A1	ANXA1	P04083
#3	Annexin A2	ANXA2	P07355
#4	CAGE	DDX53	Q86TM3
#5	Cathepsin D	CTSD	P07339
#6	CDK2	CDK2	P24941
#7	c-myc	MYC	P01106
#8	Cyclin A	CCNA2	P20248
#9	Cyclin B1	CCNB1	P14635
#10	Cyclin D1	CCND1	Q6FI00
#11	Dkk-1	DKK1	O94907
#12	GBU4-5	TDRD12	Q587J7
#13	HSP70-9B/HSPA9/GRP-75	HSPA9	P38646
#14	Hu-D	ELAVL4	P26378
#15	IMDH2	IMPDH2	P12268
#16	IMP1	IGF2BP1	Q9NZI8
#17	IMP2/IGF2BP2/p62	IGF2BP2	Q9Y6M1
#18	IMP3	IGF2BP3	O00425
#19	LAMR1	RPSA	P08865
#20	MAGEA4	MAGEA4	P43358
#21	MALAT1	MALAT1	Q9UHZ2
#22	NY-ESO-1	CTAG1B	P78358
#23	p53	TP53	P04637
#24	Paxillin	PXN	P49023-2
#25	PGAM1	PGAM1	P18669
#26	PGP9.5	UCHL1	P09936
#27	Recoverin	RCVRN	P35243
#28	SOX2	SOX2	P48431
#29	Survivin	BIRC5	O15392
#30	Ubiquilin-1	UBQLN1	Q9UMX0

3. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation 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
High Background	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
Uneven Signal	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

- Caroline J. Chapman, Graham F. Healey, Andrea Murray, et al. (2012) EarlyCDT®-Lung test: improved clinical utility through additional autoantibody assays. *Tumor Biol.* 33:1319–1326.
- Caroline J. Chapman, Alison J. Thorpe, Andrea Murray, et al. (2011) Immunobiomarkers in Small Cell Lung Cancer: Potential Early Cancer Signals. *Clin Cancer Res*; 17(6): 1474-1480.
- Stephen Lam, Peter Boyle, Graham F. Healey. (2011) EarlyCDT-Lung: An Immunobiomarker Test as an Aid to Early Detection of Lung Cancer. *Cancer Prev Res.* 4(7): 1126-1134.
- René Stempfer, Parvez Syed, Klemens Vierlinger. (2010) Tumour auto-antibody screening: performance of protein microarrays using SEREX derived antigens. *BMC Cancer.* 10:627.
- Petra Leidinger, Andreas Keller, Nicole Ludwig. (2008) Toward an early diagnosis of lung cancer: An autoantibody signature for squamous cell lung carcinoma. *Int. J. Cancer.* 123, 1631–1636.
- Chen,G., Wang,X., Yu,J., et al. (2007) Auto-antibody profiles reveal ubiquilin 1 as a humoral immune response target in lung adenocarcinoma. *Cancer Res.* 67, 3461-3467.
- Huang, R.P. (2003a). Cytokine antibody arrays: a promising tool to identify molecular targets for drug discovery. *Comb. Chem. High Throughput. Screen.* 6, 769-775.
- Huang, R.P. (2003b). Protein arrays, an excellent tool in biomedical research. *Front Biosci.* 8, D559-D576.

5. 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		

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