RayBio® Human Allergen Protein Array G3

For simultaneous detection of 82 common allergens in human serum samples, including foods, animals, mites, fungi (molds), insects, and pollen from trees, grass, and weeds.

User Manual
Version 1.0

(***Last Revised on Aug 5, 2022***)

Catalog numbers:

PAH-AGNE-G3 (Human IgE detection)
PAH-AGNG-G3 (Human IgG detection)
PAH-AGNA-G3 (Human IgA detection)

Please read manual carefully before starting experiment

RayBiotech
Empowering your proteomics

ISO 13485 CERTIFIED

www.RayBiotech.com (888) 494 – 8555 info@raybiotech.com 3607 Parkway Lane, Ste 200 Peachtree Corners, GA 30092

TABLE OF CONTENTS

I. Kit Contents and Storage

1. *Array Kit Components*

Each array kit contains the following components per 4 samples:

Item	Description	Cat. #	Size	One Glass Slide Kit
A	**Assembled Glass Slide**	AGN-G3	4 sub-arrays/slide	1 slide
B	**1,000× Biotin-Conjugated Secondary Antibody**	Vary on kit catalog numbers. See Page 6.	2 µL/vial	1 vial
C	**1,000× Cy3 Equivalent Dye Conjugated Streptavidin**	QA-CYSE	2 µL/vial	1 vial
D	**Blocking Buffer**	AA-BB-10	10 mL	1 bottle
E	**20× Wash Buffer I**	AA-WB1-30	30 mL	1 bottle
F	**20× Wash Buffer II**	AA-WB2-30	30 mL	1 bottle
G	**Adhesive Plastic Strips**		1 strip	1 strip
H	**30 ml-Centrifuge Tube**		1 tube	1 tube
I	**User Manual**	Download from www.RayBiotech.com		
J	**Analysis Tool**			
K	**Gal File**			

2. *Storage*

Upon arrival, the entire kit must be stored immediately at -20 ºC to -80 ºC until just before the experiment. If stored in this manner, the kit will retain complete activity for up to 6 months.

Once thawed, the kit must be used within 1 month. If the slide and reagents are not used immediately after thawing, store the protein array glass slide (*Item A*) and Blocking Buffer (*Item D*) at -20 ºC and store all other components (*Items B, C, E, & F*) at 4 ºC (see table *below*).

Item	Description	Storage
A	**Assembled Glass Slide**	-20 ^{o}C
B	**1,000x Biotin-Conjugated Secondary Antibody**	4 ^{o}C
C	**1,000× Fluorophore-Conjugated Streptavidin**	4 ^{o}C
D	**Blocking Buffer**	-20 ^{o}C
E	**20x Wash Buffer I**	4 ^{o}C
F	**20x Wash Buffer II**	4 ^{o}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 (Cy3 equivalent dye)

II. Introduction

1. *Assay Principle*

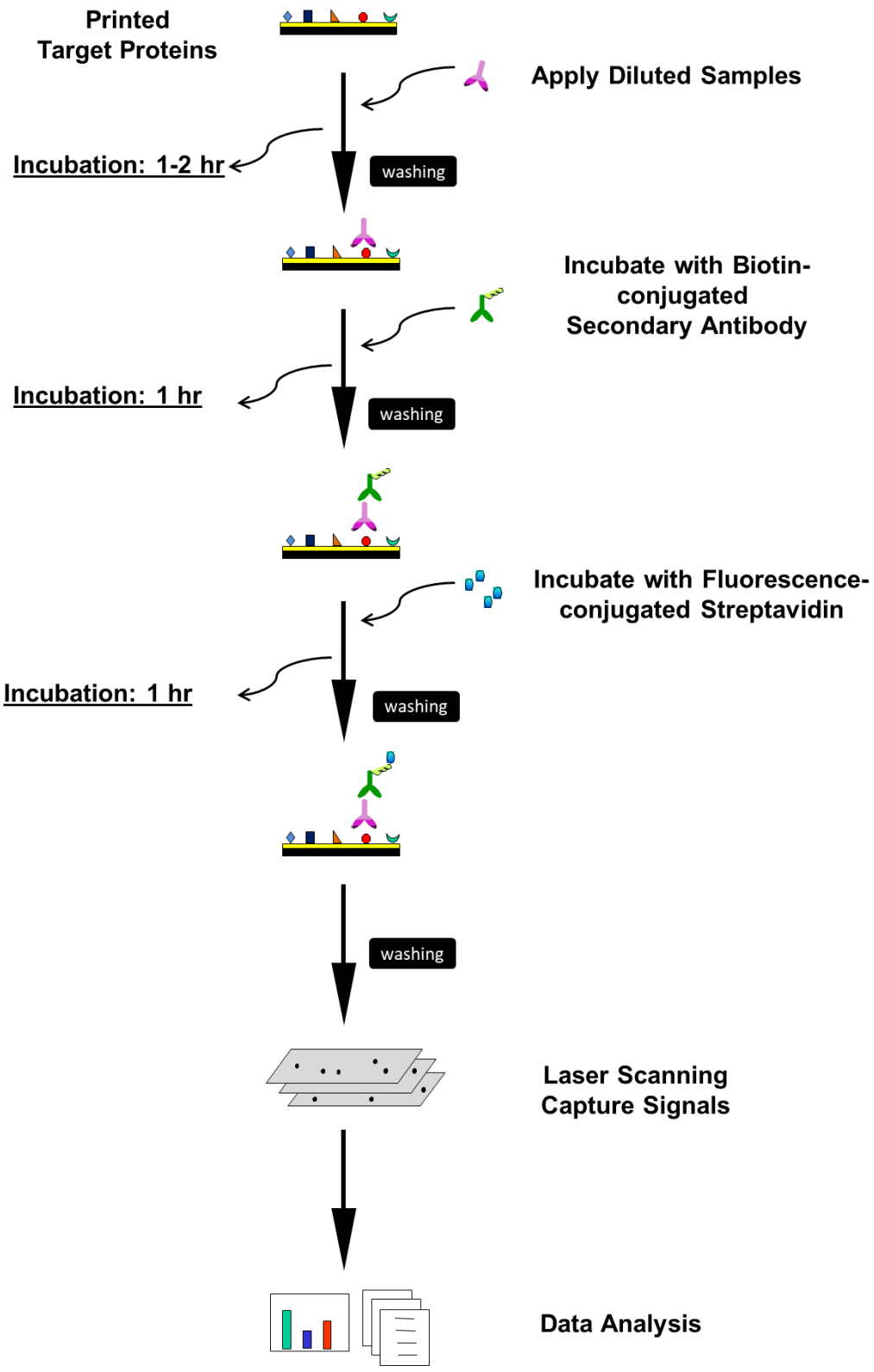
The purified recombinant allergen proteins are spotted in triplicate onto a solid glass slide surface (25 mm x 75 mm x 1 mm), and then probed for the presence of serum specific antibodies to these allergen targets. When human sera are incubated on the array, "primary" antibodies in the sample will bind to their specific target proteins. Specific primary antibody isotypes (IgE, IgA and/or IgG) from serum are then targeted by biotin-conjugated anti-human IgE, IgA and/or IgG secondary antibodies, respectively.

A fluorophore-conjugated streptavidin molecule is then added, which binds to the biotin on the secondary antibody. The fluorophore enables the detection of the immobilized primary antibody via fluorescence using a laser scanner (*see flow chart, page 6*). The fluorescence signal is proportional to the amount of immobilized antibody. Since each spot represents a unique protein that is known, the specific target(s) bound by the antibodies can be ascertained.

2. *Array Overview*

Array Format	Standard glass slide (25 mm x 75 mm x 1 mm) printed with 82 allergen proteins
Antibody Type Detected	Human IgE (Cat no. PAH-AGNE-G3) Human IgG (Cat no. PAH-AGNG-G3) Human IgA (Cat no. PAH-AGNA-G3)
Array Size	4 sub-arrays per glass slide. Each sub-array can analyze 1 sample.
Detection Method	Fluorescence (Cy3 equivalent dye) with laser scanner
Sample Volume	400 µL diluted sample per sub-array
Data Type	Semi-Quantitative
Assay Duration	< 8 hours

How it works:


Printed
Target Proteins
Apply Diluted Samples
Incubation: 1-2 hr
washing
Incubate with Biotin-conjugated Secondary Antibody
Incubation: 1 hr
washing
Incubate with Fluorescence-conjugated Streptavidin
Incubation: 1 hr
washing
washing
Laser Scanning
Capture Signals
Data Analysis

III. General Considerations

1. ***Serum Sample Preparation***

- Negative control samples (recommended): serum samples or pooled serum from healthy patients to define background signals.
- If not using fresh samples, aliquot into small tubes and freeze samples at -20 °C or 80 °C.
- Avoid repeated freeze-thaw cycles.
- Avoid using hemolyzed serum or plasma as this may interfere with protein detection.
- Always centrifuge the samples (>5,000 g for 10 minutes at 4 ^{0}C) after thawing to remove any particulates that could interfere with detection. Transfer the supernatant to new tube and keep on ice until ready to use.

2. ***Handling of 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*. Failure to do so may negatively impact the data.
- Handle the slides with powder-free gloves and in a clean environment.
- Remove reagents/samples 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 (see picture on *right*).

3. ***Incubations***

- Completely cover array area with sample or buffer during incubation steps.
- Cover the incubation chamber with adhesive strips (*Item G*) or a plastic sheet protector during incubation to avoid drying, particularly when the incubation lasts more than 2 hours or less than 70 μl 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).

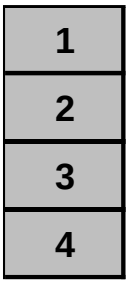
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant and aspirate the remaining liquid as shown in picture, page 7.

- Several steps such as array blocking, sample incubation, biotin-conjugated antibody incubation, and fluorescence-conjugated streptavidin incubation may be done at 4 ºC overnight. Before overnight incubations, cover the incubation chamber tightly with adhesive strips (*Item G*) to prevent evaporation.

- Protect glass slides from direct, strong light and temperatures above room temperature.

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

- The RayBio® Allergen Protein Array has 4 sub-arrays per glass slide (below). It is better to match the subarray ID numbers to your sample ID numbers. The analysis tool uses the same sample loading order (top to bottom).



- The 4-subarray glass slide has no space to print a bar code. Because of this, **the lower right corner of the printed side has a tiny green mark** using a permanent marker to ensure the slide is oriented properly. However, this green mark is covered by a black frame in its assembled configuration. After removing the frame for laser scanning, the green mark can be seen on the bottom right corner if the array side is facing up to you. **Do not use red or black colored ink** anywhere on the slide as this may negatively affect the scanned slide image and data.

IV. Protocol

The table below describes the steps and experimental outline required to perform the array detection. The whole procedure takes ~ 8 hours.

Key Step	Action	Duration
1	Equilibrate Slide	30 min
2	Air-Dry Slide	1 hour
3	Dilute Sample	< 5 min
4	Block Array	1 hour
5	Incubate Sample on Array	1 hour
6	Wash Array	40 min
7	Incubate with Biotin-Conjugated Secondary Antibody	1 hour
8	Wash Array	40 min
9	Incubate with Fluophore-Conjugated Streptavidin	1 hour
10	Wash Array, Dry Array, & Scan Array	1 hour

Before proceeding to the experiment, please refer to following dilution chart to prepare reagents. The vials containing 1,000x Biotin-Conjugated Secondary Antibody (*Item B*) and 1,000x Fluorophore-Conjugated Streptavidin (*Item C*) should be spun down briefly to collect the contents to the bottom of the vial before dilution.

Item	Description	Dilution Fold	Diluent	Temporary Storage	Shelf Life
B	**1,000x Biotin-Conjugated Secondary Antibody**	1,000	Blocking Buffer (Item D)	Fresh ice	Use immediately once diluted
C	**1,000× Fluorophore-Conjugated Streptavidin**	1,000	Blocking Buffer (Item D)	Fresh ice. Protect from light.	Use immediately once diluted
E	**20x Wash Buffer I**	20	Distilled water	Room temperature	1 week
F	**20x Wash Buffer II**	20	Distilled water	Room temperature	1 week

1. ***Blocking and Sample Incubation***

1.1 **Equilibrate Slide**: Take the kit package containing the Assembled Glass Slide (*Item A*) from the freezer. Place the **UNOPENED** package on the bench top for approximately 30 minutes and allow the Assembled Glass Slide to equilibrate to room temperature.

1.2 **Air-dry Slide**: Open package carefully and take the Assembled Glass Slide (*Item A*) out of the sleeve, but do not disassemble the Glass Slide from the chamber assembly. Peel off the cover film and let the Assembled Glass Slide air-dry in clean environment for 1 hour at room temperature.

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

1.3 **Mark Slide**: If multiple slides will be tested, you will need to distinguish one slide from another. Label the front plastic frame using tiny stickers with serial numbers. See an example of 4 sub-array slide chamber below (*left*). On the back of slide, label the very top or bottom edges of glass slides using a very fine green permanent marker with the same serial numbers (*below, right*). Don't write over the printed array area, even if it is on the back unprinted side. This slide marking can also serve to orient the slide.

Note: *Permanent marker ink can significantly interfere with fluorescent signal detection. For best results during scanning, please **DO NOT***:

- Write anywhere on the front (arrayed) side of the slide
- Write on the slide while it is wet
- Write over the arrayed well areas of the slide, as this interferes with scanning

1.4 **Block Array**: Add 400 µl of Blocking Buffer (*Item D*) into each well of the Assembled Glass Slide (*Item A*) and incubate at room temperature for 1 hour with gentle rocking. 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 along the side of well.*

1.5 **Dilute Samples**: The centrifuged samples (Section 1, page 8) should be diluted in Blocking Buffer (*Item D*), and then store on ice until ready to use. ***The optimal sample dilutions must be determined empirically by the researcher*.**

Note:

- *For human serum **IgE** detection, we normally use a 5-fold dilution (i.e.,.90 µl of centrifuged serum + 450 µl of Blocking Buffer (Item D)).*
- *For human serum **IgG** detection, we normally use a 200-fold dilution (i.e., 2.5 µl of centrifuged serum + 497.5 µl of Blocking Buffer (Item D)).*
- *For human serum **IgA** detection, we normally use a 20-fold dilution (i.e., 25 µl of centrifuged serum + 475 µl of Blocking Buffer (Item D)).*
- *Due to pipetting error and sample loss on the tubes, it is best practice to prepare more sample (e.g., 1.2 – 1.3x) than what is calculated to add to the array surface.*
- *If bulk samples are tested, we recommend performing serum dilutions in advance.*

1.6 Decant Blocking Buffer from each well completely and immediately add diluted samples.

1.7 **Incubate Samples on Array**: Load 400 µl of diluted samples into each well. Remove any bubbles from the array surfaces. Incubate arrays with gentle rocking at room temperature for 1 hour.

Note:

- *It is recommended to include control samples. For example, if testing serum from diseased patients, include serum from healthy controls.*
- *If bulk samples are tested, we recommend incubating the samples overnight at 4 ^{o}C with gentle rocking. Use the plastic adhesive strip (Item G) to seal the wells firmly.*
- *Do not let array air dry between all steps; otherwise, it will cause high background. It is recommended to handle all slides sequentially, for example, run Steps 1.6 and 1.7 for slide A first, then repeat Steps 1.6 and 1.7 for slide B, etc.*

1.8 Decant the samples from each well

1.9 Wash the wells 5 times with 500 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 after each wash step.

***Note**:* Dilute 20× Wash Buffer I (*Item E*) to 1× with distilled water. *When adding the wash buffer to the wells, avoid having the solution from one well 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 500 µl of 1× Wash Buffer II (*Item F*). Wash at room temperature with gentle shaking for 5 minutes per wash. Completely remove 1× Wash Buffer II after each wash step. Incomplete removal of the wash buffer may cause "dark spots" (i.e., the background signal is 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 Secondary Antibody Incubation***

2.1 Briefly spin down the vial of 1,000× Biotin-Conjugated Secondary Antibody (*Item B*). Add 2 ml of Blocking Buffer (*Item D*) and mix well. Spin down.

2.2 Add 400 µl of diluted Biotin-conjugated Secondary Antibody (*above*) into each well.

2.3 Incubate at room temperature for 1 hour with gentle shaking.

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. ***Fluorophore-Conjugated Streptavidin Incubation***

3.1 Briefly spin the vial containing 1,000× Cy3 Equivalent Dye-Conjugated Streptavidin (*Item C*) prior to use. Add 2 ml of Blocking Buffer (*Item D*) and mix well. Spin down.

3.2 Add 400 µl of diluted streptavidin (*above*) into each well.

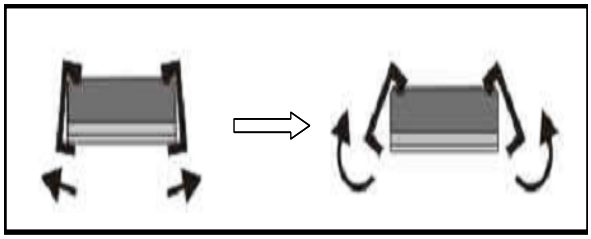
3.3 Cover the incubation chamber with aluminum foil to avoid exposure to light or perform the incubation step in a dark room.

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

3.5 Wash with 1× Wash Buffer I as described in *Step 1.9*. Then wash with 1× Wash Buffer II as described above in *Step 1.10*. Decant excess 1× Wash Buffer II from wells.

4. ***Fluorescence Detection***

4.1 Carefully disassemble the glass slide from the incubation frame and chamber by pushing the 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 green mark, which will be on the lower right corner (printed side up).



4.2 Place the whole slide in 30-ml Centrifuge Tube included in the kit (*Item H*) or a glass slide holder with the lid. Cover the tube with the aluminum foil. 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.

4.3 Wash with 1× Wash Buffer II (about 30 ml) with gentle shaking at room temperature for 10 minutes. Decant 1x Wash Buffer II.

4.4 Take the glass slide out of the wash container, and gently apply suction with a pipette to remove any water droplets. Do not touch the printed array area, only the unprinted area. Let the slide air-dry completely for 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.*

4.5 Scan slide: The array signals can be visualized through use of a compatible laser scanner capable of measuring signal in the green channel (i.e., equivalent to Cy3). Scan all slides at the same PMT. It is recommended that a higher PMT is used for low signal, and a low PMT for high signal.

V. Data Analysis

1. ***Data Extraction***

The captured array signal can be extracted with most microarray analysis software packages (e.g., GenePix, ScanArray Express, ArrayVision) 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 in 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 calculations.

The **GAL file**, which details the protein spot locations for microarray analysis software packages, can be downloaded from product page at www.Raybiotech.com

2. ***Control Systems***

Positive controls and negative controls are included on the array to assist in data normalization, array orientation determination, background evaluation, etc. These internal controls help to monitor the major assay steps, normalize data, and account for background noise. The following table describes the controls included on the array and their functions.

Controls		Function
Positive Controls	**Bio-BSA:** Biotin-Conjugated Bovine Serum Albumin (BSA)	Array orientation
		Data normalization
		Evaluate the activity of the Fluorophore-Conjugated Streptavidin (*Item C*)
	Immunoglobulin (Ig): Human IgE, IgG, and IgA	Evaluate the activity of Biotin-Conjugated Secondary Antibodies (*Item B*)
Negative Controls	**Blank:** 1x Phosphate Buffered Saline (PBS)	Evaluate the blank background level

3. ***Data Normalization***

Raw data normalization is used to compare data between sub-arrays (i.e., different samples) by accounting for the differences in signal intensities of **the positive control spots** on those arrays. The positive control (PC) is a controlled amount of biotinylated protein that is printed on the arrays in triplicate spots. The amount of signal from each PC spot is dependent on the amount of the reporter (i.e., Fluorophore-Conjugated Streptavidin) bound to biotinylated protein (Bio-BSA).

As such, any differences in the average signal of a set of PC spots from one sub-array to another sub-array will accurately reflect the signal differences between the sub-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 customer to define which array should be the reference. The normalized values are calculated as follows:

$$n\mathbf{X}s = \mathbf{X}s \times \frac{\mathbf{P}r}{\mathbf{P}s}$$

- **P***r*: the average signal value of all Bio-BSA spots on the reference array (*r*)
- **P*s***: the average signal value of all Bio-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

For example, if one sub-array that was defined as the Reference Array (*r*) had **P***r* of 40,000 and another sub-array defined as the Sample Array (*s*) had a **P***s* of 20,000, then the overall signal of the Sample Array (*s*) is half as high as the Reference Array (*r*). The equation above accounts for this discrepancy by multiplying the spot signals in the Sample Array (*s*) by 2.

4. Threshold of Significant Difference in Expression

The background signals should be subtracted from all spots, including the negative control sample's spots. The sample spot intensities across arrays should also be normalized using the positive controls as described in "Data Normalization" above. By comparing the signal intensities for each target between and among array images,

the relative differences in expression levels of each analyte between samples or groups can be determined.

Fold differences in single analyte signals across samples that are ≥1.5 may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background.

5. Analysis Tool

The extracted signal intensities from the microarray analysis software can be imported into our Excel-based Allergen Protein Array Analysis Tool (Cat. #. PAH-AGN-G3-SW). This analysis tool is simple and free to use; it can be downloaded from the product page. The RayBio® Analysis Tool software will not only assist in compiling and organizing your data, but it will also reduce your calculations to a "copy and paste" step. The Analysis Tool will help you:

- Assign your signal intensities to the array map
- Sort the target list
- Average signal intensities
- Subtract background
- Normalize the data from different samples

VI. Appendix

1. Array Target List

A total of 82 common allergen proteins (73 purified recombinant proteins and 9 chemically synthesized peptides for very small allergen proteins) were printed in triplicate on glass slides at the same protein concentration (*See table below*).

Category	Code	Allergen	Species	Common Name
I. Pet allergens:				
Small animal	A1	**Can f 1**	*Canis lupus familiaris*	Dog
Small animal	A2	**Cav p 1 ***	*Cavia porcellus*	Guinea pig
Small animal	A3	**Cav p 2 ***	*Cavia porcellus*	Guinea pig
Small animal	A4	**Fel d 1**	*Felis catus*	Cat (saliva)
Small animal	A5	**Fel d 1-A**	*Felis catus*	Cat (saliva)
Small animal	A6	**Fel d 4**	*Felis catus*	Cat (saliva)
Small animal	A7	**Ory c 4**	*Oryctolagus cuniculus*	Rabbit
Large animal	A8	**Equ c 2 ***	*Equus caballus*	Horse
Large animal	A9	**Equ c 3**	*Equus caballus*	Horse
II. Inhaled allergens:				
Fungus	A10	**Alt a 3**	*Alternaria alternata*	Mold, fungi
Fungus	A11	**Alt a 7**	*Alternaria alternata*	Mold, fungi
Fungus	A12	**Asp f 1**	*Aspergillus fumigatus*	Mold, fungi
Fungus	A13	**Asp f 15**	*Aspergillus fumigatus*	Mold, fungi
Fungus	A14	**Asp f 3**	*Aspergillus fumigatus*	Mold, fungi
Fungus	A15	**Cand a 3**	*Candida albicans*	Yeast
Fungus	A16	**Cand b 2**	*Candida boidinii*	Yeast
Fungus	A17	**Cla h 5**	*Cladosporium herbarum*	Mold, fungi
Fungus	A18	**Fus c 1**	*Fusarium culmorum*	Mold, fungi
Fungus	A19	**Mala s 5**	*Malassezia sympodialis*	Mold, fungi
Fungus	A20	**Mala s 6**	*Malassezia sympodialis*	Mold, fungi
Fungus	A21	**Pen b 26**	*Penicillium brevicompactum*	Mold, fungi
Fungus	A22	**Pen c 24**	*Penicillium citrinum*	Mold, fungi
Fungus	A23	**Pen c 3**	*Penicillium citrinum*	Mold, fungi
Insect	A24	**Api m 4 ***	*Apis mellifera*	Honeybee
Insect	A25	**Arg r 1**	*Argas reflexus*	Pigeon tick
Insect	A26	**Bla g 5**	*Blattella germanica*	German cockroach
Insect	A27	**Bla g 6**	*Blattella germanica*	German cockroach
Insect	A28	**Blo t 5**	*Blomia tropicalis*	Storage mite
Insect	A29	**Der f 1**	*Dermatophagoides farinae*	House dust mite
Insect	A30	**Der f 2**	*Dermatophagoides farinae*	House dust mite
Insect	A31	**Der m 1 ***	*Dermatophagoides microcera*	House dust mite
Insect	A32	**Der p 1**	*Dermatophagoides pteronyss*	House dust mite
Insect	A33	**Der p 2**	*Dermatophagoides pteronyss*	House dust mite
Insect	A34	**Gly d 2**	*Glycyphagus domesticus*	House itch mite
Insect	A35	**Per a 1**	*Periplaneta americana*	American cockroach
Insect	A36	**Per a 6**	*Periplaneta americana*	American cockroach
Insect	A37	**Pol a 5**	*Polistes annularis*	Wasp
Insect	A38	**Tyr p 13**	*Tyrophagus putrescentiae*	Mold mite
Insect	A39	**Tyr p 2**	*Tyrophagus putrescentiae*	Mold mite

III. Food allergens:				
Animal	A40	**Bos d 2**	*Bos domesticus*	Beef
Animal	A41	**Cha f 1**	*Charybdis feriata*	Crab
Animal	A42	**Cyp c 1**	*Cyprinus carpio*	Carp
Animal	A43	**Gal d 2**	*Gallus gallus*	Chicken egg white
Animal	A44	**Met e 1**	*Metapenaeus ensis*	Shrimp
Crop	A45	**Api g 4**	*Apium*	Celery
Crop	A46	**Cap a 2**	*Capsicum annuum*	Bell pepper
Crop	A47	**Dau c 4**	*Daucus carota*	Carrot
Crop	A48	**Gly m 3**	*Glycine max*	Soybean
Crop	A49	**Gly m 4**	*Glycine max*	Soybean
Crop	A50	**Lyc e 1**	*Solanum lycopersicum*	Tomato
Crop	A51	**Ory s 12**	*Oryza sativa subsp. japonica*	Rice
Crop	A52	**Zea m 12**	*Zea mays*	Corn, maize
Crop	A53	**Zea m 14**	*Zea mays*	Corn, maize
Fruit	A54	**Act d 5**	*Actinidia deliciosa*	Kiwi
Fruit	A55	**Ana c 1**	*Ananas comosus*	Pineapple
Fruit	A56	**Cit l 3 ***	*Citrus limon*	Lemon
Fruit	A57	**Cit s 2**	*Citrus sinensis*	Orange
Fruit	A58	**Lit c 1**	*Litchi chinensis*	Litchi, Lychee
Fruit	A59	**Mal d 1**	*Malus domestica*	Apple
Fruit	A60	**Mus xp 1**	*Musa acuminata*	Banna
Fruit	A61	**Pru ar 1**	*Prunus armeniaca*	Apricot
Fruit	A62	**Pru p 1**	*Prunus persica*	Peach
Fruit	A63	**Pru p 4**	*Prunus persica*	Peach
Fruit	A64	**Pyr c 4**	*Pyrus communis*	Pear
Nut	A65	**Ara h 7**	*Arachis hypogaea*	Peanut
Nut	A66	**Hel a 2**	*Helianthus annuus*	Sunflower
Nut	A67	**Hel a 3**	*Helianthus annuus*	Sunflower
Nut	A68	**Pru du 4**	*Prunus dulcis*	Almond
IV. Environmental allergens:				
Tree	A69	**Aln g 4**	*Alnus glutinosa*	European alder
Tree	A70	**Bet v 1**	*Betula verrucos*	European white birch
Tree	A71	**Bet v 2**	*Betula verrucosa*	European white birch
Tree	A72	**Hev b 5**	*Hevea brasiliensis*	Rubber tree (latex)
Tree	A73	**Hev b 6 ***	*Hevea brasiliensis*	Rubber tree (latex)
Tree	A74	**Hev b 8**	*Hevea brasiliensis*	Rubber tree (latex)
Grass, weed	A75	**Amb a 10**	*Ambrosia artemisiifolia*	Short ragweed
Grass, weed	A76	**Amb a 8**	*Ambrosia artemisiifolia*	Short ragweed
Grass, weed	A77	**Amb t 5 ***	*Ambrosia trifida*	Giant ragweed
Grass, weed	A78	**Art v 3 ***	*Artemisia vulgaris*	Mugwort
Grass, weed	A79	**Art v 4**	*Artemisia vulgaris*	Mugwort
Grass, weed	A80	**Che a 2**	*Chenopodium album*	Pigweed
Grass, weed	A81	**Che a 3**	*Chenopodium album*	Pigweed
Grass, weed	A82	**Phl p 2**	*Phleum pratense*	Timothy grass

* Chemically synthesized peptides

2. Array Map

Each sub-array is printed in a 21-column x 22-row format (*below*). All proteins were printed in triplicate. Human IgE, IgG and IgA, biotinylated bovine serum albumin (Bio-BSA) are positive control spots whereas the 1x Phosphate Buffered Saline (PBS) are negative control (Blank) spots.

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12	Column 13	Column 14	Column 15
Row 1	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	A17	A17	A17	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	A52	A52	A52	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)
Row 2	Bio-BSA (2)	Bio-BSA (2)	Bio-BSA (2)	A18	A18	A18	A35	A35	A35	A53	A53	A53	A70	A70	A70
Row 3	A1	A1	A1	A19	A19	A19	A36	A36	A36	A54	A54	A54	A71	A71	A71
Row 4	A2	A2	A2	A20	A20	A20	A37	A37	A37	A55	A55	A55	A72	A72	A72
Row 5	A3	A3	A3	A21	A21	A21	A38	A38	A38	A56	A56	A56	A73	A73	A73
Row 6	A4	A4	A4	A22	A22	A22	A39	A39	A39	A57	A57	A57	A74	A74	A74
Row 7	A5	A5	A5	A23	A23	A23	A40	A40	A40	A58	A58	A58	A75	A75	A75
Row 8	A6	A6	A6	A24	A24	A24	A41	A41	A41	A59	A59	A59	A76	A76	A76
Row 9	A7	A7	A7	A25	A25	A25	A42	A42	A42	A60	A60	A60	A77	A77	A77
Row 10	A8	A8	A8	A26	A26	A26	A43	A43	A43	A61	A61	A61	A78	A78	A78
Row 11	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	A27	A27	A27	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	A62	A62	A62	A79	A79	A79
Row 12	A9	A9	A9	A28	A28	A28	A44	A44	A44	A63	A63	A63	A80	A80	A80
Row 13	A10	A10	A10	A29	A29	A29	A45	A45	A45	A64	A64	A64	A81	A81	A81
Row 14	A11	A11	A11	A30	A30	A30	A46	A46	A46	A65	A65	A65	A82	A82	A82
Row 15	A12	A12	A12	A31	A31	A31	A47	A47	A47	A66	A66	A66	Blank	Blank	Blank
Row 16	A13	A13	A13	A32	A32	A32	A48	A48	A48	A67	A67	A67	Human IgE	Human IgE	Human IgE
Row 17	A14	A14	A14	A33	A33	A33	A49	A49	A49	A68	A68	A68	Human IgG	Human IgG	Human IgG
Row 18	A15	A15	A15	A34	A34	A34	A50	A50	A50	A69	A69	A69	Human IgA	Human IgA	Human IgA
Row 19	A16	A16	A16	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	A51	A51	A51	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)

3. *Reference List*

1. 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.

2. Huang, R.P. Protein arrays, an excellent tool in biomedical research. Front Biosci. 2003b. 8, D559-576.

3. Casali P, Schettino EW. Structure and function of natural antibodies. Curr Top Microbiol Immunol. 1996. 210:167-179.

4. Chen, C.M., et al. The role of cats and dogs in asthma and allergy–a systematic review. Int. J Hyg Environ Health 2010. 213:1-31.

5. Custis, N.J., et al. Quantitative measurement of airborne allergens from dust mites, dogs, and cats using an ion-charging device. Clin Exp Allergy 2003. 33:986-991.

6. Richardson G, et al. How is the indoor environment related to asthma?: literature review. Journal of Advanced Nursing. 2005. Vol 52(3), 328-339.

7. Diane R. Gold, Environmental Tobacco Smoke, Indoor Allergens, and Childhood Asthma. 2000. Environmental Health Perspectives. Vol. 108, Supplement 4, 643-651.

8. Tan, Y.W., et al. Structures of Two Major Allergens, Bla g 4 and Per a 4, from Cockroaches and Their IgE Binding Epitopes. Journal of Biochemistry. 2009. Vol 284(5), 3148-3157.

9. Arruda, L.K., Chapman, M.D. The role of cockroach allergens in asthma. Current Opinion in Pulmonary Medicine 2001. 7:14-19.

4. ***Troubleshooting Guide***

Problem	Potential Causes	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 at 4 ^{o}C
	Serum, protein or antibody concentrations are too low	Dilute sample less, concentrate sample, or add more sample volume to well. If adding more sample to well, ensure that the samples do not cross-contaminate neighboring wells.
	Improper storage of kit	Store kit at suggested temperature; Don't freeze/thaw the slide
High Background	Excess protein or antibody	Further dilute serum, protein or antibody
	Excess streptavidin	Further dilute streptavidin
	Overexposure	Lower the laser power
	Dust	Minimize dust in work environment before starting experiment
	Slide dried out between steps	Take additional precautions to prevent slides from drying out during experiment
	Dark spots	Completely remove wash buffer after each wash step
	Insufficient washing	Increase wash time and use more wash buffer. Wash slide in Wash Buffer I (*Item E*) overnight at 4 ^{o}C.
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 were not completely covered by reagent	Prepare more reagent and completely cover arrays with solution

Note:

RayBio® is the trademark of RayBiotech Life, Inc.

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 Life, Inc.

Under no circumstances shall RayBiotech Life, Inc. be liable for any damages arising out of the use of the 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 Life, Inc.'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.