RayBio® SARS-CoV-2 Spike Protein Array

For IgM and IgG Detection in Serum & Plasma

Peptide-based array on a glass slide

User Manual
(*Revised April 6, 2020*)

Catalog Number: PAH-SASP-G1-16

Please read manual carefully before starting experiment

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

RayBiotech
Empowering your proteomics

Tel: 1-888-494-8555, 770-729-2992
Fax: 1-770-206-2393
Website: www.raybiotech.com
E-mail: info@raybiotech.com

Array Overview

Array Format	Glass slide printed with synthesized peptides
Antibody Type Detected	Human IgG and IgM
Array Size	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

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I. Kit Contents and Storage

This manual provides the guidelines and instructions for detecting IgG and IgM antibody isotypes in serum or plasma using the RayBio® SARS-CoV-2 Spike Protein Array.

1. ***Array Kit Components***

Each array kit contains the following components (16 samples).

Item	Description	Cat. #	Size	One Glass Slide Kit
A	**Assembled Glass Slide**	PAH-SASP-G1-16	16 sub-arrays	1 slide (16 sub-arrays)
B1	**1,000× Biotin-conjugated Anti-Human IgG**	AHG-BIO-01	1.5 µL/vial	2 vials
B2	**1,000× Biotin-conjugated Anti-Human IgM**	AHM-BIO-01	1.5 µL/vial	2 vials
C	**1,500× Cy3 equivalent dye conjugated Streptavidin**	AA-HRP-G	1 µL/vial	2 vials
D	**Blocking Buffer**	AA-BB	10 mL	1 bottle
E	**20× Wash Buffer I**	AA-WB1	30 mL	1 bottle
F	**20× Wash Buffer II**	AA-WB2	30 mL	1 bottle
G	**Adhesive Plastic Strips**		1 strip	1 strip
H	**30 ml-Centrifuge Tube**		1 tube	1 tube

Notes:

- *Items B1, B2 & 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 ºC to -80 ºC until just before the experiment. At -20 ºC to -80 ºC, 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 ºC and all other components (*Items B, C, E, & F*) should be stored at 4 ºC (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 ºC
B1	1,000× Biotin-conjugated Anti-Human IgG	4 ºC
B2	1,000× Biotin-conjugated Anti-Human IgM	4 ºC
C	1,500× HiLyte 555 Streptavidin	4 ºC
D	Blocking Buffer	-20 ºC
E	20× Wash Buffer I	4 ºC
F	20× Wash Buffer II	4 ºC
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)

The RayBio® SARS-CoV-2 Spike Protein Array Analysis Tool (Cat. #. PAH-SASP-G1-SW) is an Excel-based software specific for this array that is very helpful in analyzing and organizing the array data. To receive the free software, please send your invoice number related to your RayBio® SARS-CoV-2 Spike Protein Array purchase to *info@raybiotech.com*.

II. Introduction

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

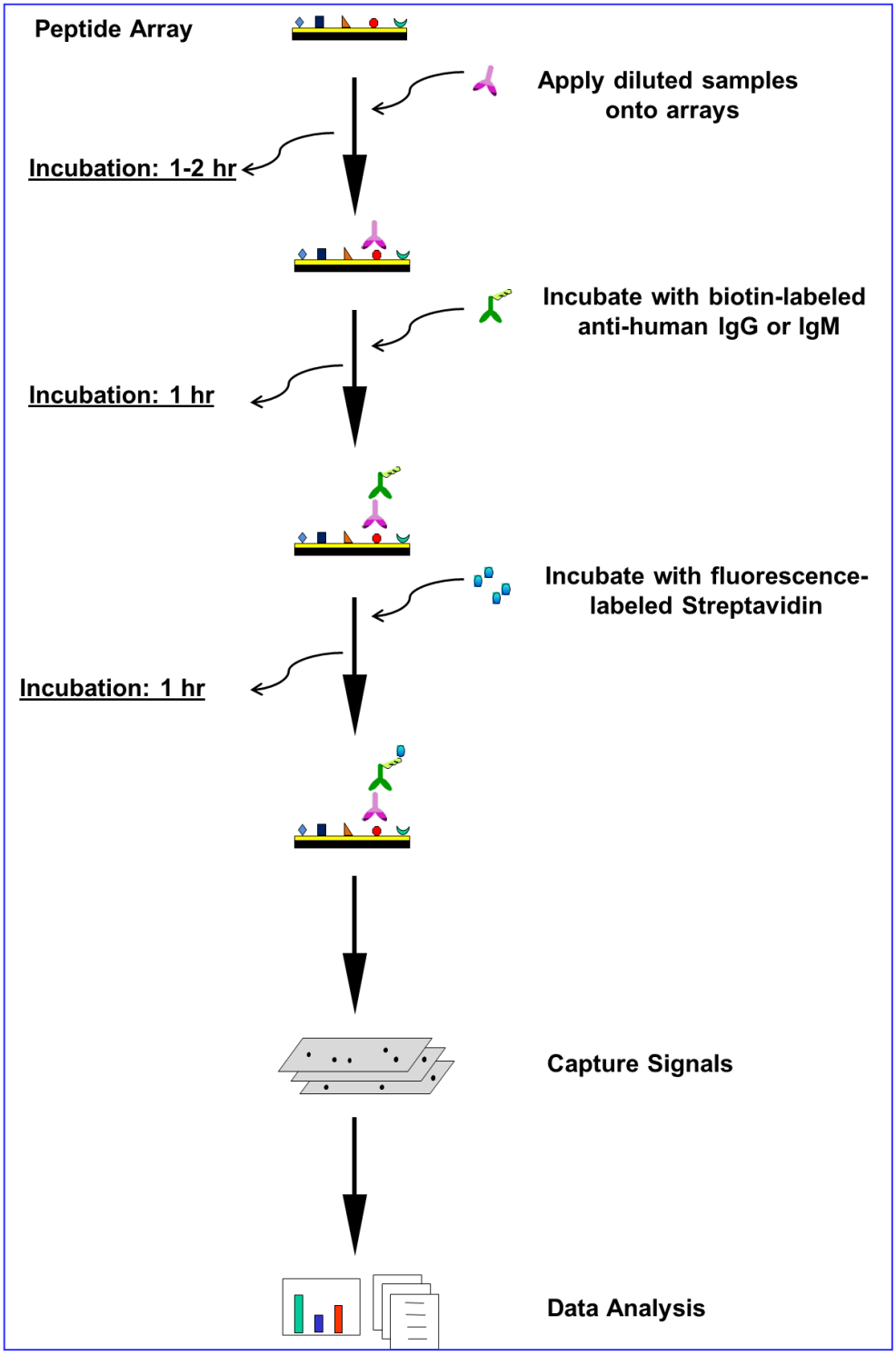
Microarray-based antibody detection is the foundation of high throughput antibody screening in human samples. In this assay (see the flow chart on the *next page*), peptides representing the SARS-CoV-2 Spike protein are arrayed in quadruplicate on a glass slide surface. The assay includes multiple positive and negative controls to monitor each incubation step.

Serum or other fluid samples are diluted and incubated on the protein arrays, during which serological IgG or IgM antibodies bind to their specific peptide epitope. After washing to remove unbound antibodies and other materials in the fluid, the arrays are incubated with biotin-conjugated anti-human IgG or IgM. The secondary antibody is then bound by a fluorescence dye conjugated streptavidin molecule to enable the detection of immobilized antibody via fluorescence using a laser scanner. The fluorescence signal is proportional to the amount of immobilized antibody. Since each spot represents a known unique peptide, the specific epitopes bound by the antibodies can be ascertained.

2. ***Array Features***

- Low sample consumption: as little as 2 µl of original serum sample required per array.
- High sensitivity: both biotin-streptavidin pair and fluorescent detection enable the most sensitive assay available to measure serological IgG or IgM.
- 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.
- Easy data interpretation.

How Peptide Array Works:



III. Overview and General Considerations

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

- **Negative control samples (recommended)**: serum samples or pooled serum pool from healthy patients 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 ^{0}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 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. Failure to do so may negatively impact the data.
- 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 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 *above*.

- Several incubation steps such as blocking, sample incubation, biotin-conjugated antibody incubation, or fluorescence-conjugated streptavidin incubation may be done at 4 ºC 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***

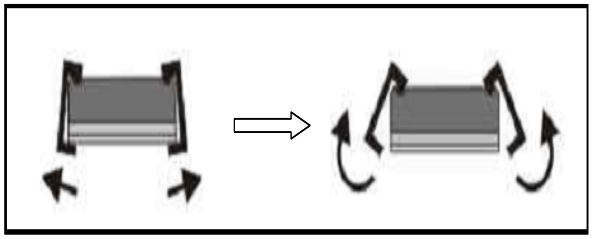
- The RayBio® SARS-CoV-2 Spike Protein Array is available in 16 sub-arrays per glass slide.

- The 16-subarray glass slide has no place to print a bar code. Because of this, mark the bottom right corner of the printed side with a tiny green mark using a permanent marker to ensure the slide is oriented properly. Do not use red or black colored ink anywhere on the slide as this may negatively affect the scanned slide image and data.

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***

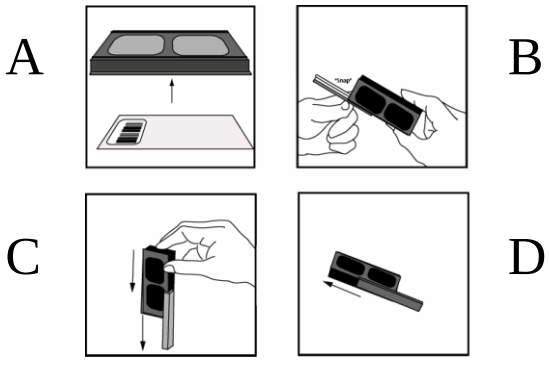
After finishing your experiment, if you need to repeat any of the incubation or wash steps, you must first re-assemble the glass slides into the incubation chamber. Do this by following the steps as shown in the figures below. To avoid breaking the printed glass slide, it is recommended that you first practice assembling the device with a regular blank standard glass histology (or microscope) 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. The whole procedure takes ~ 8 hours. *If bulk samples are tested, we recommend incubating the samples overnight at 4 ^{0}C with gentle rocking*.

Step	Action	Time
1	Equilibrate kit to room temperature	30 min
2	Air-dry glass slides	1 hour
3	Array blocking	1 hour
4	Sample incubation	1 hour
5	Array washing	40 min
6	Biotin-conjugated anti-human IgG or IgM 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	To be determined by customer. For IgG, we normally use 200-fold dilutions.	Blocking Buffer (Item D)	Mix 1 µl serum with 199 µl of Blocking Buffer. Mix and spin down.	Fresh ice	Use immediately once diluted
B1	**1,000× 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
B2	**1,000× Biotin-conjugated Anti-Human IgM**	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	**1,500× Cy3 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	**20× Wash Buffer I**	20	Distilled water	Dilute with 19-times distilled water. Mix well.	Room temperature	1 week
F	**20× 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 approximately 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 and 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 incubate 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 ^{0}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 samples on ice.

Note: Optimal dilution factors for each sample must be determined by the investigator.

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 condition 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* 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 or IgM Incubation

2.1 Briefly spin down the vial of 1,000× Biotin-conjugated Anti-human IgG (*Item B1*) or Anti-human IgM (*Item B2)*. 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 or IgM 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*

1.1 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.

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

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

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

1.5 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.

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 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.

2. ***Control Systems***

To help data analysis, multiple positive controls and negative controls are included on the array to assist in data normalization, array orientation determination, background evaluation, etc. Besides the normal serum controls, the comprehensive internal controls help to monitor the major assay steps, data normalization, and background reading. The following table describes all of the controls included on the array 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 and IgM (serial diluted)	Array orientation
		Data normalization
		Evaluate the activity of biotin-labeled anti-human IgG antibody
	Recombinant SARS-CoV-2 Spike and N proteins	Full length target proteins to check the presence of specific antibodies in samples
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***

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** 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 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:

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

- **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

Sample spot intensities should subtract background signals from the negative control spots and, if included, serum samples from healthy patients. 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.

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 the laser scanner can simply be imported into our analysis tool, RayBio® SARS-CoV-2 Spike Protein Array Analysis Tool (Cat. #. PAH-SASP-G1-SW). This analysis tool is simple and free to use. This Excel-based software will not only assist in compiling and organizing your data, but will also reduce your calculations to a “copy and paste” step. The analysis tool will help you:

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

To receive the free software, please send your invoice number related to your RayBio® SARS-CoV-2 Spike Protein Array purchase to *info@raybiotech.com*.

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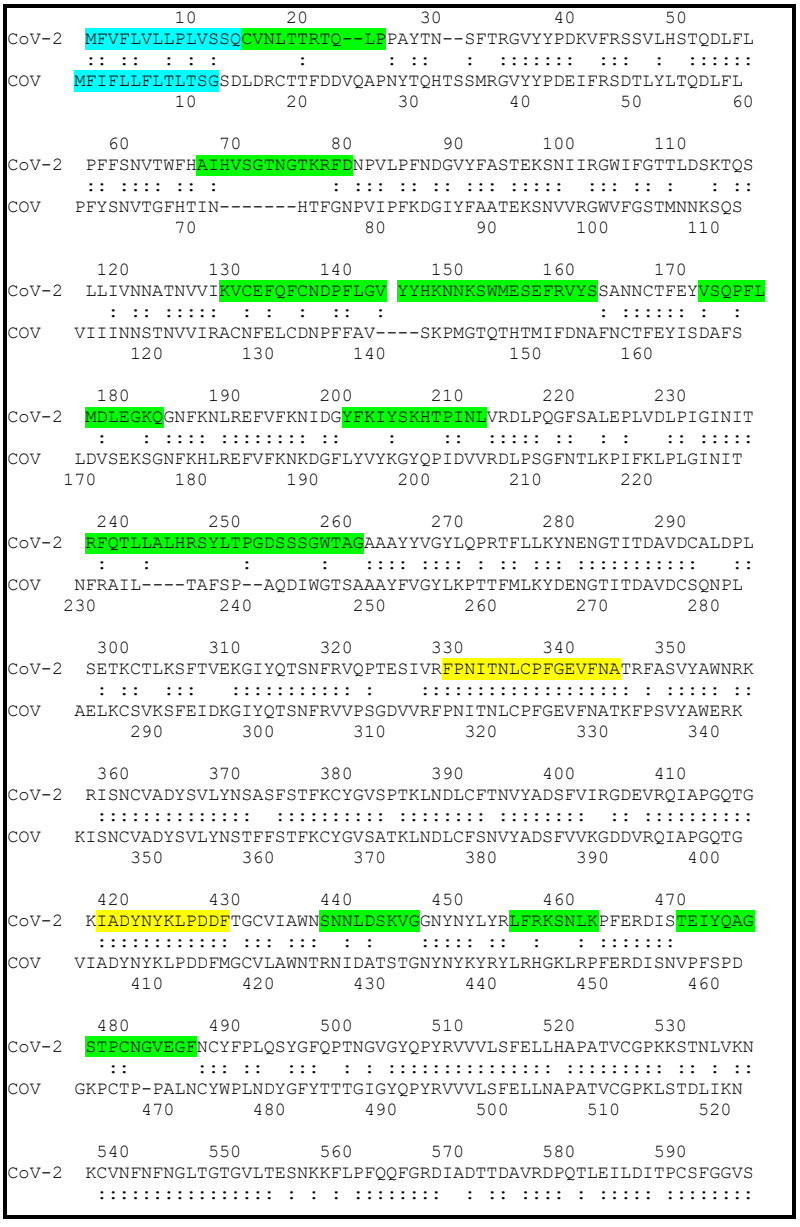
VI. Appendix

1. Array Target List

Spike proteins of SARS-CoV (responsible for the SARS outbreak in 2003) and SARS-CoV-2 (responsible for the SARS outbreak in 2019) have high sequence similarity. Based on the sequence alignment of the spike protein S1 subunits between SARS-CoV (Protein accession #. P59594) and SARS-CoV-2 (Protein accession #. QHD43416), 11 unique peptides to SARS-CoV-2 (*Figure below, in green*) and 3 conserved peptides between two proteins (*Figure below, in yellow*) were chemically synthesized (*see table next page*).



The chemically synthesized peptide sequences of spike proteins:

	Target	Sequence	Species
Peptides	WUSP-1	CVNLTTRTQLP	SARS-CoV-2
	WUSP-2	AIHVSGTNGTKRFD	
	WUSP-3	KVCEFQFCNDPFLGV	
	WUSP-4	YYHKNNKSWMESEFRVYS	
	WUSP-5	VSQPFLMDLEGKQ	
	WUSP-6	YFKIYSKHTPINL	
	WUSP-7	RFQTLLALHRSYLTPGDSSSGWTAG	
	WUSP-8	SNNLDSKVG	
	WUSP-9	LFRKSNLK	
	WUSP-10	TEIYQAGSTPCNGVEGF	
	WUSP-11	QTQTNSPRRARSVASQ	
	WuSa-1	SYECDIPIGAGICASY	SARS-CoV-1, SARS-CoV-2
	WuSa-2	FPNITNLCPFGEVFNA	
	WuSa-3	IADYNYKLPDDF	
Control proteins	230-30162	S1 subunit, RBD	SARS-CoV-2
	230-30163	S2 subunit, full length	
	230-30164	N protein, full length	

2. Array Map

This array is printed in the following format (*Table below*). The Biotin-BSA are positive control spots whereas the 1x PBS are negative control spots. Purified recombinant glycosylated SARS-CoV-2 S1 subunit protein, RBD domain (Cat. 230-30162), S2 subunit protein (Cat. 230-30163), and N protein (Cat. 230-30164) expressed from mammalian cells were included as controls.

	1	2	3	4	5	6	7	8	
1	Biotin-BSA (1)	Biotin-BSA (1)	Biotin-BSA (1)	Biotin-BSA (1)	Human IgG	Human IgG	Human IgG	Human IgG	1
2	Biotin-BSA (2)	Biotin-BSA (2)	Biotin-BSA (2)	Biotin-BSA (2)	WUSP-10	WUSP-10	WUSP-10	WUSP-10	2
3	WUSP-1	WUSP-1	WUSP-1	WUSP-1	WUSP-11	WUSP-11	WUSP-11	WUSP-11	3
4	WUSP-2	WUSP-2	WUSP-2	WUSP-2	WuSa-1	WuSa-1	WuSa-1	WuSa-1	4
5	WUSP-3	WUSP-3	WUSP-3	WUSP-3	WuSa-2	WuSa-2	WuSa-2	WuSa-2	5
6	WUSP-4	WUSP-4	WUSP-4	WUSP-4	WuSa-3	WuSa-3	WuSa-3	WuSa-3	6
7	WUSP-5	WUSP-5	WUSP-5	WUSP-5	1x PBS	1x PBS	1x PBS	1x PBS	7
8	WUSP-6	WUSP-6	WUSP-6	WUSP-6	S1, RBD	S1, RBD	S1, RBD	S1, RBD	8
9	WUSP-7	WUSP-7	WUSP-7	WUSP-7	S2	S2	S2	S2	9
10	WUSP-8	WUSP-8	WUSP-8	WUSP-8	N protein	N protein	N protein	N protein	10
11	WUSP-9	WUSP-9	WUSP-9	WUSP-9	1x PBS	1x PBS	1x PBS	1x PBS	11
12	Human IgM	Human IgM	Human IgM	Human IgM	Biotin-BSA (2)	Biotin-BSA (2)	Biotin-BSA (2)	Biotin-BSA (2)	12
	1	2	3	4	5	6	7	8	

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 dying 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***

- F Wu, et al. A new coronavirus associated with human respiratory disease in China. Nature. 579, 265–269 (2020).
- N Dong, et al. Genomic and protein structure modelling analysis depicts the origin and infectivity of 2019-nCoV, a new coronavirus which caused a pneumonia outbreak in Wuhan, China. bioRxiv (2020).
- M Hoffmann, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 181, 1–10 (2020).
- W Li et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 426, 450–454 (2003).

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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