RayBio® Porcine (pig) Cytokine Protein Array G1

For simultaneous detection of 51 essential pig cytokine proteins to investigate protein-protein interactions, antibody specificity, autoantibodies, and small molecule-protein interactions

User Manual
Version 1.0

(***Last Revised on Aug 17, 2022***)

Catalog numbers:

PAP-CYTG-G1 (Porcine IgG detection)

PAP-CYTA-G1 (Porcine IgA detection)

Please read manual carefully before starting experiment

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I. Kit Contents and Storage

1. *Array Kit Components*

Each array kit contains the following components per 16 samples:

Item	Description	Cat. #	Size	One Glass Slide Kit
A	**Assembled Glass Slide**	PIG-G1	16 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 ºC
B	**1,000x Biotin-Conjugated Secondary Antibody**	4 ºC
C	**1,000× Fluorophore-Conjugated Streptavidin**	4 ºC
D	**Blocking Buffer**	-20 ºC
E	**20x Wash Buffer I**	4 ºC
F	**20x 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)

II. Introduction

1. ***Assay Principle***

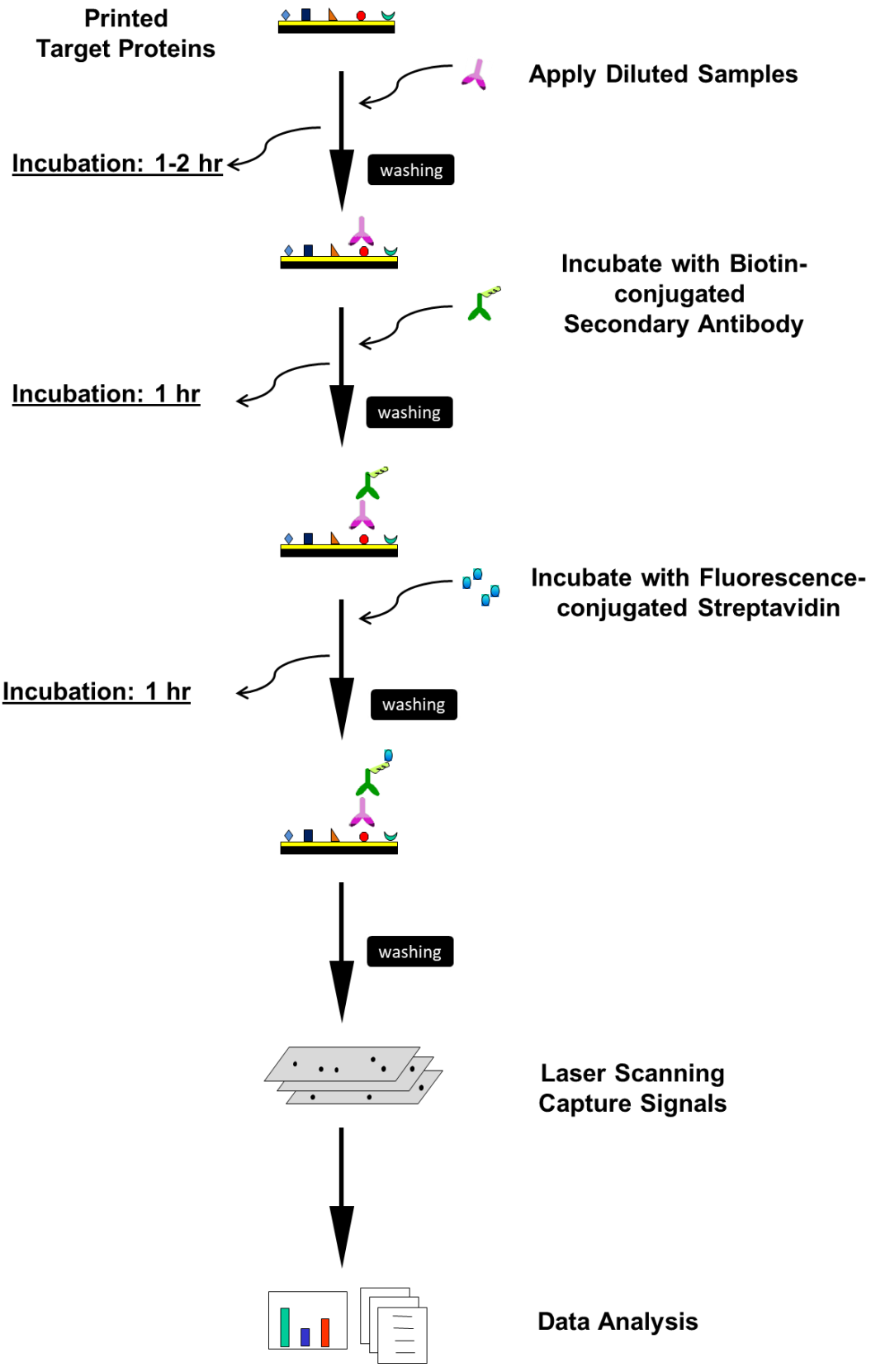
The purified recombinant pig cytokine proteins from bacteria and HEK293 cells are spotted in triplicate onto a solid glass slide surface (25 mm x 75 mm x 1 mm), and then probed for the presence of serum specific antibodies, purified antibodies, or other binders. When samples are incubated on the array, "primary" antibodies in the sample will bind to their specific target proteins. Specific primary antibody isotypes (IgG and/or IgA) from samples are then targeted by biotin-conjugated anti-porcine IgG and/or IgA 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. The kits can be applied in screening protein-protein interactions, monitoring the presence of autoantibodies, determining antibody specificity, and/or detecting small molecule-protein interactions.

2. ***Array Overview***

Array Format	Standard glass slide (25 mm x 75 mm x 1 mm) printed with 51 recombinant pig proteins
Antibody Type Detected	Porcine IgG (Cat no. PAP-CYTG-G1) Porcine IgA (Cat no. PAP-CYTA-G1)
Array Size	16 sub-arrays per glass slide. Each sub-array can analyze 1 sample.
Detection Method	Fluorescence (Cy3 equivalent dye) with laser scanner
Sample Volume	100 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

3. Array Applications

Since RayBio® protein arrays have multiple applications, which require different procedures. Only some examples of the potential uses of our protein array are given here.

1. **Detection of protein-protein interactions**. The kit can be used to screen novel protein-protein interactions, validate the previously known protein-protein interactions, and investigate the molecule interaction conditions.
2. **Characterization of antibodies**. The kit can be used to test the specificity of an antibody for research and therapeutic antibody development and find out the potential cross-reaction to other proteins.
3. **Target identification**. The kit can be used to screen the small molecule-protein interaction for target identification, drug discovery and toxicity study.
4. **Detection of autoantibodies**. The kit can be used to detect and characterize autoantibodies from serum and other body fluids.
5. **Detection of protein-DNA interaction**. In some cases, the kit can be used to detect DNA binding proteins.

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® Porcine Protein Array G1 has 16 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*).

1	**2**
3	**4**
5	**6**
7	**8**
9	**10**
11	**12**
13	**14**
15	**16**

- The 16-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 following protocol described how to detect IgG and IgA from pig serum. For other applications, such as protein-protein interactions, antibody specificity, auto-antibodies, and small molecule-protein interactions, the following protocol needed to be further modified to fit to your assays. Please contact us how to help you develop a specific 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.

Front Side Back Side

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 100 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 pig serum **IgG** detection, we normally use a 200-fold dilution (i.e., 1 µl of centrifuged serum + 199 µl of Blocking Buffer (Item D)).*
- *For pig serum **IgA** detection, we normally use a 20-fold dilution (i.e., 6 µl of centrifuged serum + 114 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 100 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 100 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 100 µl of diluted streptavidin (*above*) into each well.

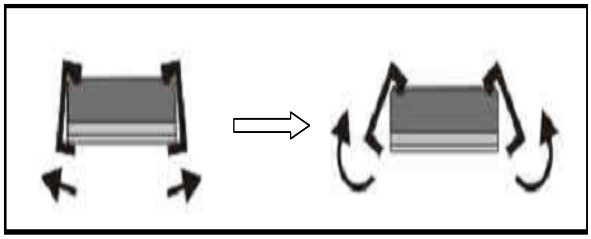
3.3 Cover the incubation chamber with aluminum foil to avoid exposure to light or perform the incubation step in a dark room.

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): Pig 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 Porcine Cytokine Protein Array G1 Analysis Tool (Cat. #. PAP-CYT-G1-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 51 essential porcine cytokine proteins expressed from bacteria and HEK293 cells were printed in triplicate on glass slides at the same protein concentration (*See table below*).

Code	Short Description	Gene Symbol	UniProt Accession #
P1	Angiogenin	ANG	P31346
P2	Apolipoprotein M	APOM	Q2LE37
P3	BDNF	BDNF	P14082
P4	Beta-NGF	NGF, NGFB	Q29074
P5	CCL4	CCL4	Q711P4
P6	CCL8	CCL8	P49873
P7	Complement C5a	C5	P01032
P8	CRP	CRP	O19062
P9	CSF	CSF2	Q29118
P10	CTLA4	CTLA4	Q9MYX7
P11	Cystatin A	Cystatin-A1	Q28988
P12	Cystatin B	CSTB	Q29290
P13	Cytochrome c	CYCS	P62895
P14	Erythropoietin (EPO)	EPO	P49157
P15	FABP1	FABP1	P49924
P16	FABP2	FABP2	Q45KW7
P17	FABP3	FABP3	O02772
P18	Ferritin light chain	FTL	P19133
P19	G-CSF	CSF3	O02837
P20	IGF-I	IGF1	P16545
P21	IGF-II	IGF2	P23695
P22	IL-10	IL10	Q29055
P23	IL-13	IL13	Q95J68
P24	IL-17A	IL17A	Q60I29
P25	IL-18	IL18	O19073
P26	IL-1a	IL1A	P18430
P27	IL-1b	IL1B	P26889
P28	IL-1ra	IL1RN	Q29056
P29	IL-2	IL2	P26891

P30	IL-21	IL21	Q76LU6
P31	IL-4	IL4	Q04745
P32	IL-6	IL6	P26893
P33	IL-7	IL7	Q9N2G6
P34	Inhibin alpha chain	INHA	P04087
P35	Inhibin beta B chain	INHBB	P04088
P36	Interferon alpha-1	IFNA	P49879
P37	Interferon gamma	IFNG	P17803
P38	Leptin	LEP	Q29406
P39	MCP-1	CCL2	P42831
P40	MIF	MIF	P80928
P41	Myoglobin	MB	P02189
P42	NT-3	NTF3	Q06AV0
P43	Serum amyloid P (SAP)	APCS	O19063
P44	TFF2	TFF2	P01359
P45	TGFA	TGFA	Q06922
P46	TGFb1	TGFB1	P07200
P47	TGFb2	TGFB2	P09858
P48	TGFB3	TGFB3	P15203
P49	TIMP-1	TIMP1	P35624
P50	TNF-a	TNFa	P23563
P51	Troponin C	TNNC1	P63317

2. Array Map

Each sub-array is printed in a 15-column x 12-row format (*below*). All proteins were printed in triplicate. Porcine 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)	P11	P11	P11	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	P33	P33	P33	P45	P45	P45
Row 2	Bio-BSA (2)	Bio-BSA (2)	Bio-BSA (2)	P12	P12	P12	P22	P22	P22	P34	P34	P34	P46	P46	P46
Row 3	P1	P1	P1	P13	P13	P13	P23	P23	P23	P35	P35	P35	P47	P47	P47
Row 4	P2	P2	P2	P14	P14	P14	P24	P24	P24	P36	P36	P36	P48	P48	P48
Row 5	P3	P3	P3	P15	P15	P15	P25	P25	P25	P37	P37	P37	P49	P49	P49
Row 6	P4	P4	P4	P16	P16	P16	P26	P26	P26	P38	P38	P38	P50	P50	P50
Row 7	P5	P5	P5	P17	P17	P17	P27	P27	P27	P39	P39	P39	P51	P51	P51
Row 8	P6	P6	P6	P18	P18	P18	P28	P28	P28	P40	P40	P40	Blank	Blank	Blank
Row 9	P7	P7	P7	P19	P19	P19	P29	P29	P29	P41	P41	P41	**Pig IgG**	**Pig IgG**	**Pig IgG**
Row 10	P8	P8	P8	P20	P20	P20	P30	P30	P30	P42	P42	P42	**Pig IgA**	**Pig IgA**	**Pig IgA**
Row 11	P9	P9	P9	P21	P21	P21	P31	P31	P31	P43	P43	P43	Blank	Blank	Blank
Row 12	P10	P10	P10	Bio-BSA (1)	Bio-BSA (1)	Bio-BSA (1)	P32	P32	P32	P44	P44	P44	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.

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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:

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