Quantibody® Human Serum Abundant Protein Array 4

Quantitative measurement of 40 Human Serum Abundant serum proteins

Catalog #: QAH-SAP-4

User Manual
Last revised August 25, 2021

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

ISO 13485 Certified

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (40)	Azurocidin, BLMH, C1q R1, C1qTNF1, C2, Cathepsin A, Cathepsin D, Cathepsin X/Z/P, Aminopeptidase N, CD35, Contactin-4, CPA1, CPM, DNER, Endorepellin, Fetuin B, Hepassocin, LEKTI, MAGP-2/MFAP5, MCAM, MMR, Neuropilin-1, PAM, Park7, PCSK9, PLUNC, PRCP, PRTN3, P-Selectin, PSP94, S100A6, SAA, SLPI, SPARCL1, Tenascin C, Thioredoxin-1, TIM-4, Trypsin Pan, Thrombospondin-4, VSIG4 *See Section IX for Array Map*
Format	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
Detection Method	Fluorescence. Go to www.RayBiotech.com/Scanners for a list of compatible laser scanners.
Sample Volume	50 - 100 µl per array
Reproducibility	CV <20%
Assay Duration	6 hours

II. Introduction

Human serum is an ideal biological sample for biomarker discovery due to its homogeneous characteristics, non-invasive nature, and immediate availability for most studies. While most of the serological cytokines can be detected with our Quantibody arrays with a simple 2x dilution, some analytes are abundant proteins which need further sample dilution. We have developed three human specific serum abundant protein arrays (QAH-SAP-1, 2, 3, 4) to account for the high prevalence of these factors in serum samples. Array 1 is ideal for 10x serum dilution, Arrays 2 and 4 are for 100x sample dilution, while Array 3 is targeted for two dilutions: 1000x and 100,000x dilutions. These three arrays expand on RayBiotech?s ability to discover and quantify serological biomarkers.

The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent assay (ELISA). In this method, target protein is immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the enzyme complex can then be visualized through the use of a substrate that produces a detectable signal. While this traditional method works well for a single protein, the overall procedure is time consuming and

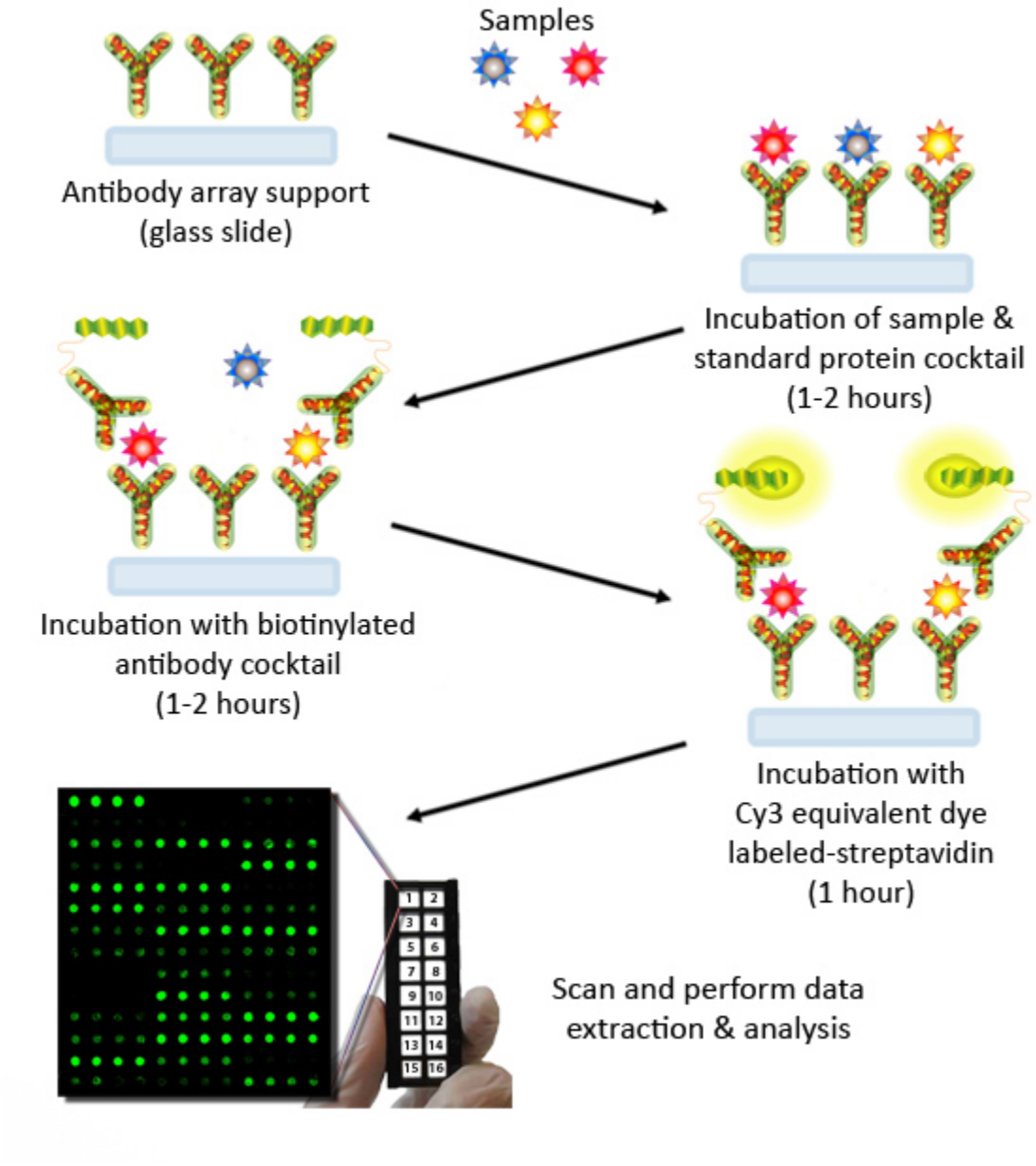
requires a relatively high volume of sample. Thus, conservation of precious small sample quantities becomes a challenging task. Innovations in microarray technology over the last decade have addressed this problem. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which have now been widely applied in the research community with hundreds of peer reviewed publications, including top-tier journals such as *Cell* and *Nature*.

The Quantibody® array, our multiplexed sandwich ELISA-based quantitative array platform, enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and the high throughput of arrays. Like a traditional sandwich-based ELISA, it uses a pair of cytokine specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target cytokine is trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different epitope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-conjugated Cy3 equivalent dye, using a laser scanner. Unlike the traditional ELISA, Quantibody products use an array format. By arraying multiple cytokine specific capture antibodies onto a glass support, quantitative, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is divided into 16 wells of identical cytokine antibody arrays. Each antibody, together with the positive controls is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples on one slide. Four slides can be nested into a tray, which matches a standard microplate footprint and allows for automated robotic high throughput process of 64 arrays simultaneously. For cytokine quantification, the array specific cytokine standards, whose concentration has been predetermined, are provided to generate a standard curve for each cytokine. In a real experiment, standard cytokines and samples will be assayed in each array simultaneously through a sandwich ELISA procedure. By comparing signals from unknown samples to the standard curve, the cytokine concentration in the samples will be determined.

Quantibody® array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Our current high density Quantibody kits allow scientists to quantitatively determine the concentration of 1000 human, 200 mouse, and 67 rat cytokines in a single experiment. This is not only one of the most efficient products on the market for cytokine quantification, but makes it more affordable for quantification of large number of proteins. Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for drug and biomarker discovery.

III. How It Works



IV. Materials Provided

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	QAH-SAP-4 S	Human Serum Abundant Protein Array 4 Glass Slide	1	2
2	QA-SDB	Quantibody® Sample Diluent	15 ml	
3	AA-WB1-30ML	20X Wash Buffer I	2 x 30 ml	3 x 30 ml
4	AA-WB2-30ML	20X Wash Buffer II	30 ml	
5	QAH-SAP-4 -STD	Human Serum Abundant Protein Array 4 Lyophilized Standard Mix**	1 Vial	
6	QAH-SAP-4 B	Human Serum Abundant Protein Array 4 Biotinylated Antibody Cocktail	1-25 µl	2 x 1-25 µl
7	QA-CY3E	Cy3 equivalent dye-conjugated Streptavidin	5 µl	2 x 5 µl
8	QA-SWD	Slide Washer/Dryer	1 x 30 ml Tube	
9	QA-ADH	Adhesive Film	1	2

* 4 slide kits are comprised of 2 separate 2 slide kits.
** See Section X for detailed cytokine concentrations after reconstitution.

V. Storage

Upon receipt, all components should be stored at -20°C. The kit will retain activity for up to 6 months. Once thawed, the glass slide, standard mix, antibody cocktail and dye-conjugated Streptavidin should be kept at -20°C. All other components may be stored at 4°C. The entire kit should be used within 6 months of purchase.

VI. Additional Materials Required

- Benchtop rocker or orbital rocker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- 1.5 ml Polypropylene microcentrifuge tubes

VII. General Considerations

A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contains cytokines.
- Each array needs 100 µl of total sample volume. To avoid matrix effects, we recommend using a minimum of 2-fold sample dilution of culture media, body fluids, or 0.5-1mg/ml total protein for lysates, after a 5-fold to 10-fold dilution to minimize the effects of any detergent(s). Please be aware, more sample volume is required for combination arrays. For example, the minimum sample volume for a 10-array kit is 500 µl, or 500 µg lysate.
- The suggested serum/plasma dilution for this array is: 100x

B. Handling Glass Slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with powder free gloves.
- Handle glass slide/s in clean environment.
- Permanent marker ink can significantly interfere with fluorescent signal detection. To help distinguish one slide from another, you may make a small marking (such as a number or a star) along the top or bottom edge, using a green or blue ultra-fine point Sharpie® brand marker. This can also serve to orient the slide. 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
    - Use red or black colored ink anywhere on the slide
    - Write over the arrayed well areas of the slide, as this interferes with scanning.

C. Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rocking or rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µl of sample or reagent is used.

- Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

VIII. Protocol

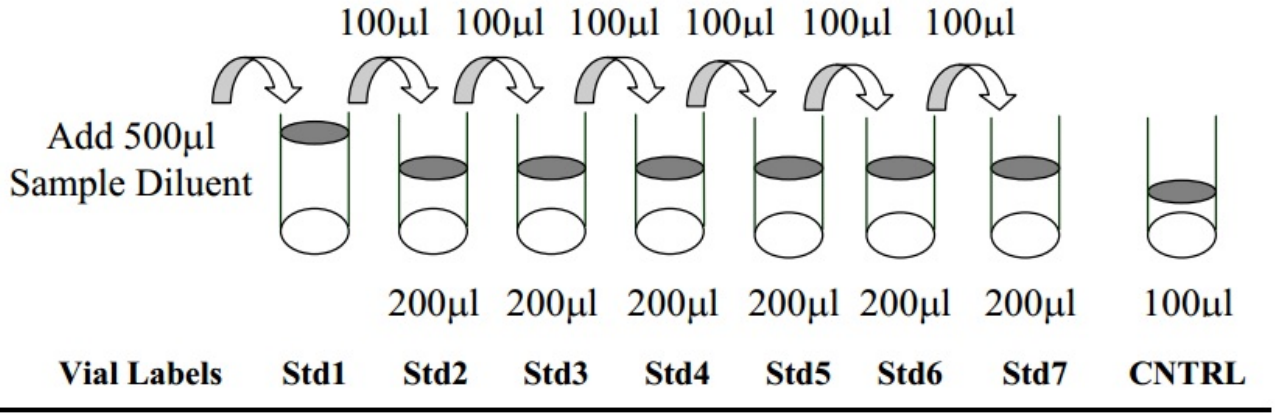
A. Completely Air Dry The Glass Slide

1. Take out the glass slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag, peel off the cover film, and let it air dry for another 1-2 hours.

Incomplete drying of slides before use may cause the formation of "comet tails," thin directional smearing of antibody spots.

B. Prepare Cytokine Standard Dilutions

There is only one vial of standard provided in the two-slide kit, which is enough for making two standard curves. Reconstitute the lyophilized standard within one hour of usage. If you must use the standard for two different days, store only the Std1 dilution at -80°C.



2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500 µl Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1.

3. Label 6 clean microcentrifuge tubes as Std2 to Std7. Add 200 µl Sample Diluent to each of the tubes.

4. Pipette 100 µl Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100 µl Std2 to tube Std3 and so on.

5. Add 100 µl Sample Diluent to another tube labeled as CNTRL. Do not add standard cytokines or samples to the CNTRL tube, which will be used as negative control. For best results, include a set of standards in each slide.

Since the starting concentration of each cytokine is different, the serial concentrations from Std1 to Std7 for each cytokine are varied which can be found in Section X.

C. Blocking & Incubation

6. Add 100 µl Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.

7. Decant buffer from each well. Add 100 µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour.

Longer incubation time is preferable for higher signals. This step may be done overnight at 4°C.

We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 250 µg/ml-1 mg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation, especially if less than 70 ul of sample or reagent is used.

8. Wash:

- Decant the samples from each well, and wash 5 times (5 min each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with H2O.

- *(Optional for Cell and Tissue Lysates)* Put the glass slide with frame into a box with 1X Wash Buffer I (cover the whole glass slide and frame with Wash Buffer

I), and wash at room temperature with gentle rocking for 20 min.

- Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 150 µl of 1X Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step. Dilute 20X Wash Buffer II with H2O.

Incomplete removal of the wash buffer in each wash step may cause "dark spots," the background signals higher than the spots.

D. Incubation with Biotinylated Antibody Cocktail & Wash

9. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.

10. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour.

Longer incubation time is preferable for higher signals and backgrounds

11. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I and then 2 times with 150 µl of 1x Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

E. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash

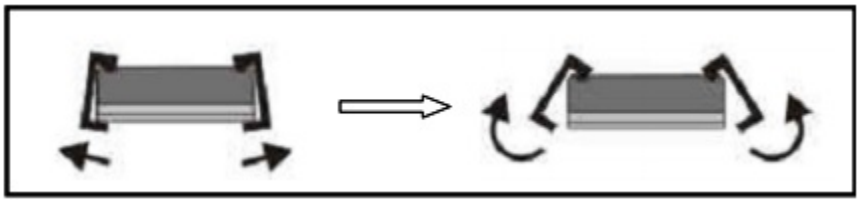
12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

13. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.

14. Decant the samples from each well, and wash 5 times (5 mins each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

F. Fluorescence Detection

15. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.



Be careful not to touch the surface of the array side.

16. Place the slide in the Slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) and gently shake at room temperature for 5 minutes.

17. Remove water droplets completely by gently applying suction with a pipette to remove water droplets. Do not touch the array, only the sides.

    You may also dry the glass slide by a compressed N_2 stream.

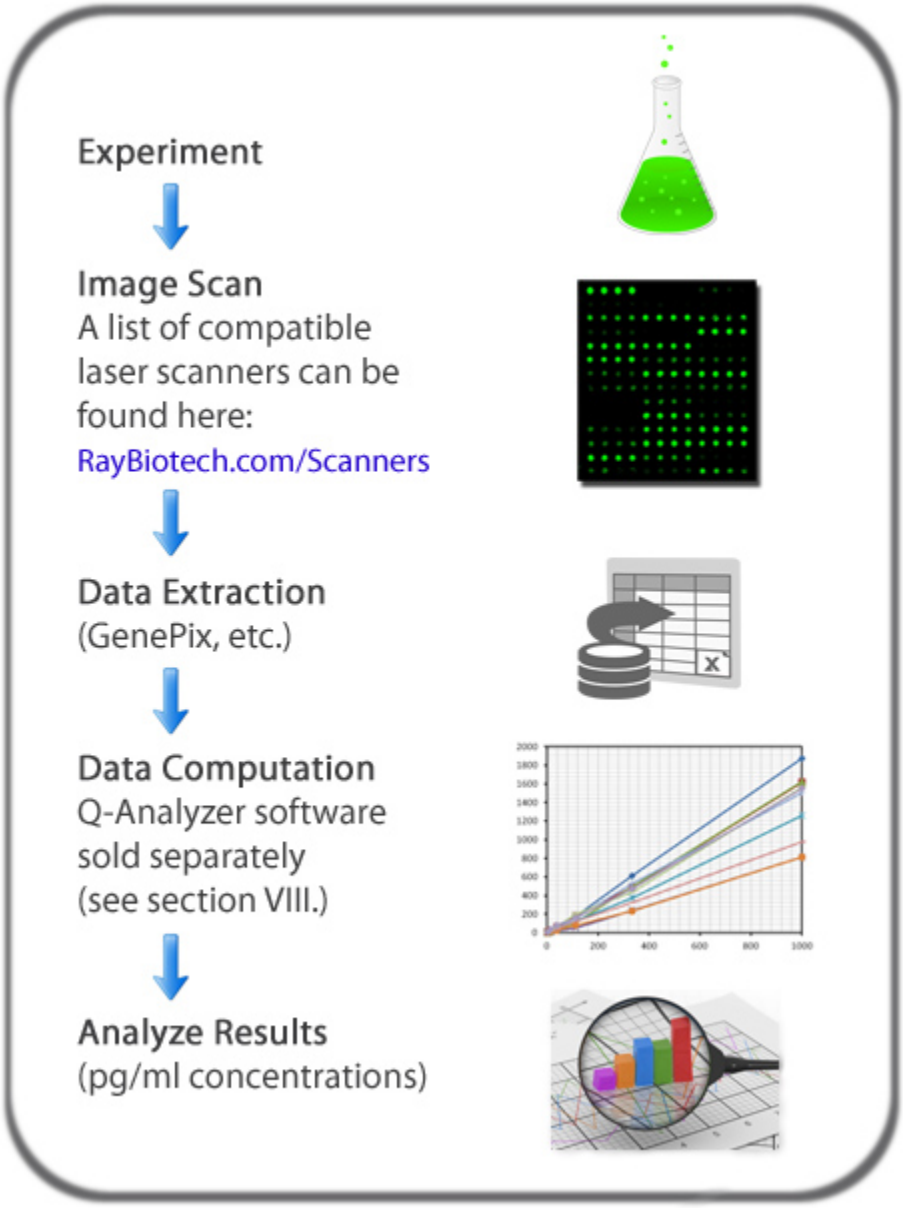
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains unsaturated.

In case the signal intensity for different cytokine varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal cytokines, and a low PMT for high signal cytokines.

G. Data Analysis

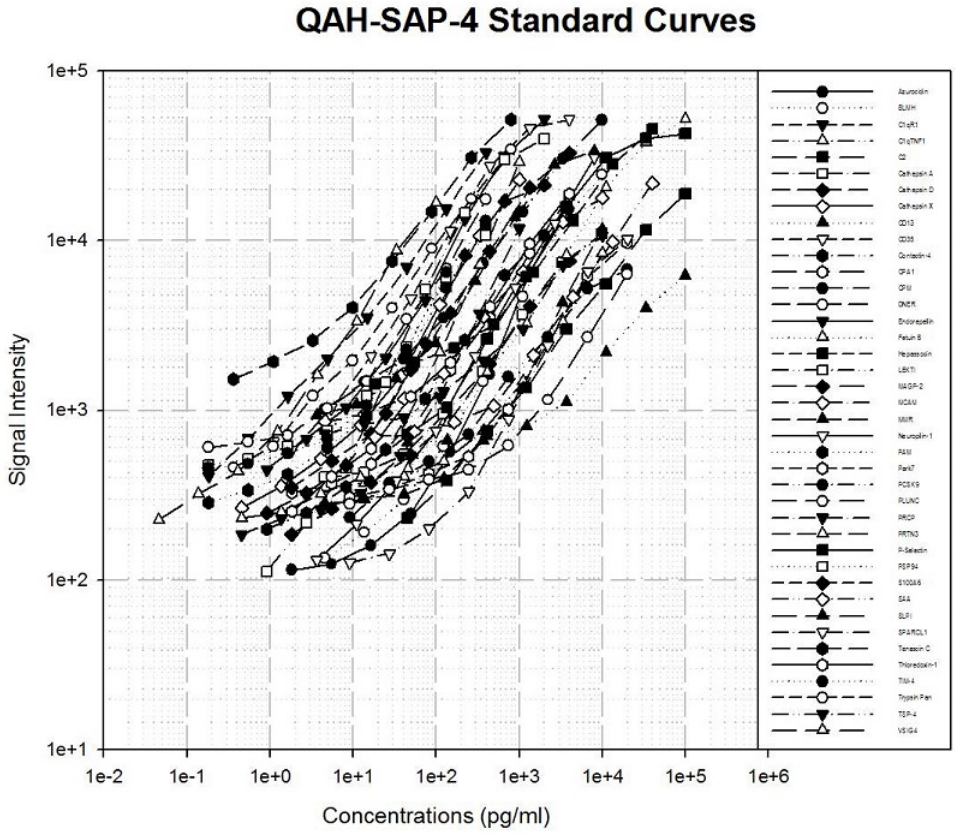
19. Data extraction can be done using the GAL file that is specific for this array (QAH-SAP-4) along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). The GAL file can be found on the product web page under the 'Files' tab.

    Need help analyzing all that data? All RayBiotech array analysis tools are now free to download! Just like the GAL file, you can find this 'Q-Analyzer' tool on the product web page under the 'Files' tab. More information can be found in Section XII.



IX. Array Map & Standard Curves

(hSAP-4 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Azurocidin			
B	BLMH				C1q R1				C1qTNF1			
C	C2				Cathepsin A				Cathepsin D			
D	Cathepsin X				CD13				CD35			
E	Contactin-4				CPA1				CPM			
F	DNER				Endorepellin				Fetuin B			
G	Hepassocin				LEKTI				MAGP-2			
H	MCAM				MMR				Neuropilin-1			
I	PAM				Park7				PCSK9			
J	PLUNC				PRCP				PRTN3			
K	P-Selectin				PSP94				S100A6			
L	SAA				SLPI				SPARCL1			
M	Tenascin C				Thioredoxin-1				TIM-4			
N	Trypsin Pan				TSP-4				VSIG4			



X. Standard Concentrations

After reconstitution, the lyophilized cytokine standard mix contains the following concentrations for each antigen included.

QAH-SAP-4 Serial Standard Concentrations (pg/ml)

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
Azurocidin	2	5	16	49	148	444	1333	4,000
BLMH	5	14	41	123	370	1111	3333	10,000
C1q R1	0	1	2	5	15	44	133	400
C1qTNF1	5	14	41	123	370	1111	3333	10,000
C2	46	137	412	1235	3704	11111	33333	100,000
Cathepsin A	5	14	41	123	370	1111	3333	10,000
Cathepsin D	2	5	16	49	148	444	1333	4,000
Cathepsin X	0	1	4	12	37	111	333	1,000
CD13	46	137	412	1235	3704	11111	33333	100,000
CD35	2	5	16	49	148	444	1333	4,000
Contactin-4	1	3	8	25	74	222	667	2,000
CPA1	2	5	16	49	148	444	1333	4,000
CPM	5	14	41	123	370	1111	3333	10,000
DNER	0	1	2	5	15	44	133	400
Endorepellin	1	3	8	25	74	222	667	2,000
Fetuin B	46	137	412	1235	3704	11111	33333	100,000
Hepassocin	18	55	165	494	1481	4444	13333	40,000
LEKTI	1	3	8	25	74	222	667	2,000
MAGP-2	1	3	8	25	74	222	667	2,000
MCAM	5	14	41	123	370	1111	3333	10,000
MMR	5	14	41	123	370	1111	3333	10,000
Neuropilin-1	4	11	33	99	296	889	2667	8,000
PAM	0	1	2	5	15	44	133	400
Park7	9	27	82	247	741	2222	6667	20,000
PCSK9	9	27	82	247	741	2222	6667	20,000
PLUNC	9	27	82	247	741	2222	6667	20,000
PRCP	0	1	4	12	37	111	333	1,000
PRTN3	0	1	4	12	37	111	333	1,000
P-Selectin	46	137	412	1235	3704	11111	33333	100,000
PSP94	0	1	2	5	15	44	133	400
S100A6	2	5	16	49	148	444	1333	4,000
SAA	18	55	165	494	1481	4444	13333	40,000
SLPI	4	11	33	99	296	889	2667	8,000
SPARCL1	9	27	82	247	741	2222	6667	20,000
Tenascin C	0	1	3	10	30	89	267	800
Thioredoxin-1	2	5	16	49	148	444	1333	4,000
TIM-4	0	1	2	5	15	44	133	400
Trypsin Pan	0	1	3	10	30	89	267	800
TSP-4	5	14	41	123	370	1111	3333	10,000
VSIG4	0	0	0	1	4	11	33	100

XI. Spiking & Recovery

The antibody pairs used in the kit have been tested to recognize their specific antigen. The spiking recovery rates of each protein in some common fluids are listed in the following tables.

QAH-SAP-4 media spike-in recovery rate

(pg/ml)	Spike-in	Serum	Serum+Ag	Serm%	CM	CM+Ag	CM%
Azurocidin	2,000	145	2,299	108%	0	2,622	131%
BLMH	5,000	45	4,303	85%	0	5,543	111%
C1q R1	200	38	256	109%	0	201	100%
C1qTNF1	5,000	839	5,911	101%	0	5,138	103%
C2	50,000	6,916	62,783	112%	0	65,125	130%
Cathepsin A	5,000	46	4,309	85%	3	2,925	58%
Cathepsin D	2,000	145	2,735	130%	2	1,747	87%
Cathepsin X	500	31	592	112%	0	516	103%
CD13	50,000	1,600	62,330	121%	0	50,476	101%
CD35	2,000	1	1,648	82%	0	1,439	72%
Contactin-4	1,000	1	993	99%	0	782	78%
CPA1	2,000	24	1,732	85%	2	1,536	77%
CPM	5,000	1	6,334	127%	0	6,186	124%
DNER	200	5	165	80%	0	194	97%
Endorepellin	1,000	5	1,343	134%	0	1,337	134%
Fetuin B	50,000	461	52,493	104%	2	54,537	109%
Hepassocin	20,000	480	27,974	137%	42	24,273	121%
LEKTI	1,000	2	724	72%	1	732	73%
MAGP-2	1,000	7	937	93%	1	922	92%
MCAM	5,000	338	4,378	81%	22	5,340	106%
MMR	5,000	433	5,834	108%	0	6,223	124%
Neuropilin-1	4,000	109	2,359	56%	8	3,530	88%
PAM	200	14	210	98%	1	185	92%
Park7	10,000	161	7,658	75%	267	7,401	71%
PCSK9	10,000	20	10,769	107%	7	9,111	91%
PLUNC	10,000	0	11,384	114%	155	10,002	98%
PRCP	500	24	517	99%	10	424	83%
PRTN3	500	9	665	131%	1	666	133%
P-Selectin	50,000	99	49,676	99%	747	37,713	74%
PSP94	200	2	231	115%	1	260	130%
S100A6	2,000	56	1,126	53%	0	1,638	82%
SAA	20,000	0	15,276	76%	38	16,940	85%
SLPI	4,000	66	3,811	94%	12	3,383	84%
SPARCL1	10,000	775	10,222	94%	49	9,381	93%
Tenascin C	400	7	553	136%	6	571	141%
Thioredoxin-1	2,000	13	1,679	83%	3	1,445	72%
TIM-4	200	6	297	145%	4	284	140%
Trypsin Pan	400	2	223	55%	0	202	51%
TSP-4	5,000	973	7,971	140%	128	4,776	93%
VSIG4	50	0	34	68%	0	67	135%

XII. Quantibody® Q-Analyzer

The Q-Analyzer tool can be downloaded from the 'Files' tab on the product web page for this array: **QAH-SAP-4**.

The Q-Analyzer is an array specific, Excel-based program. It is much more than a simple calculation macro; it performs sophisticated data analysis (see below for description).

Key features:

- Simplicity: Easy to operate and requires no professional training. With a simple copy and paste process, the cytokine concentration is determined.
- Outlier Marking & Removing: The software can automatically mark and remove the outlier spots for more accurate data analysis
- Normalization: The program allows for intra- and inter-slide normalization for large numbers of samples.
- Two Positive Controls: The program utilizes the two positive controls in each array for normalization.
- Two Analytical Algorithms: Users can choose either linear regression or log-log algorithms to meet their analytical needs.
- Two Data Outputs: standard curves and digital concentration.
- User Intervention: The program allows for user manual handling of outliers and other analytical data.
- Lower and Upper Limits Determination: The program automatically marks out the values below or above the detection range.
- Standard Deviation: The program outputs the standard deviations of the quadruplicate spots for data accuracy.
- Analytical Tips: Q-Analyzer analysis tips are included in the program.

XIII. 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 time	Increase incubation time or change sample incubation step to overnight
	Too low protein concentration in sample	Lessen dilution or do not dilute sample. Concentrate sample if necessary.
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Decrease amount of rocking during incubations. check for bubble formation and remove bubbles.
	Arrays are not completed covered by reagent	Completely cover arrays with solution for all required steps.
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer and other solutions into neighboring wells.
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard well at the room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions.
	Inadequate detection	Increase laser power so the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated.
	Use freeze-thawed cytokine standards	Always use new cytokine standard vial for new set of experiment. Discard any leftover.
High background	Overexposure	Lower the PMT or signal gain.
	Dark spots	Completely remove wash buffer in each wash step.
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Work in clean environment
	Slide is allowed to dry out	Don't dry out slides during experiment.

XIV. Select Quantibody® Publications

1. Zeng Q., et al. The functional behavior of a macrophage/fibroblast co-culture model derived from normal and diabetic mice with a marine gelatin-oxidized alginate hydrogel. Biomaterials. 2010 Aug;31(22):5772-81. doi: 10.1016/j.biomaterials.2010.04.022.
   Species: Mouse
2. Toh H, Wang W, Chia W, Kvistborg P, Sun Li,et al. Clinical Benefit of Allogeneic Melanoma Cell Lysate-Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients.Clin Cancer Res. 2009;15(24):7726-7736
   Species: Human
   Sample Type: Plasma
3. Du Y, Wei X, He Y, Wei G, Hampel H, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. Alzheimer Dementia. 2008;4(4 Suppl):T484 (Abstract P2-380).
   Species: Human
   Sample Type: Plasma
4. Jonnalagadda D., et al. Platelet secretion is kinetically heterogeneous in an agonist-responsive manner. December 20, 2012; Blood: 120 (26). http://dx.doi.org/10.1182/blood-2012-07-445080
   Species: Human
   Sample Type: Conditioned Media
5. Vargas-Inchaustegui D., Hogg A., Tulliano G., et al.CXCL10 Production by Human Monocytes in Response to Leishmania braziliensis Infection. Infect. Immun. January 2010 vol. 78 no. 1 301-308
   Species: Human
   Sample Type: Serum
6. Zhai Y, Zhong Z, Chen C-YA, Xia Z, Song L, Blackburn MR, Shyu A-B. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. Mol Cell Biol. 2008; 28(24):7414-7426.
   Species: Human
7. Huggenberger R., et al. Stimulation of lymphangiogenesis via VEGFR-3 inhibits chronic skin inflammation. J Exp Med. 2010 Sep 27;207(10):2255-69. doi: 10.1084/jem.20100559.
   Species: Mouse
   Sample Type: Tissue Lysate
8. Jurk D., Wilson C., Passos J., et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nature Communications 2, Article number: 4172. doi:10.1038/ncomms5172
   Species: Mouse
   Sample Type: Conditioned Media
9. Bethunaickan, R., Sahu, R., Liu, Z., Tang, Y. T., Huang, W., Edegbe, O., Tao, H., Ramanujam, M., Madaio, M. P. and Davidson, A. (2012), Anti-tumor necrosis factor alpha treatment of interferon-alpha-induced murine lupus nephritis reduces the renal macrophage response but does not alter glomerular immune complex formation. Arthritis & Rheumatism, 64: 3399-3408. doi: 10.1002/art.34553
   Species: Mouse
   Sample Type: Tissue Lysate
10. Hou T., Li Z., Luo F., Xie Z., Wu X., Xing J., Dong S., Xu J. A composite demineralized bone matrix e Self assembling peptide scaffold for enhancing cell and growth factor activity in bone marrow. Biomaterials, Available online 19 April 2014. [Epub ahead of print]
    Species: Mouse
    Sample Type: Tissue Lysate
11. Feng W., Madajka M., Kerr B., Mahabeleshwar G., White S., Byzova T. A novel role for platelet secretion in angiogenesis: mediating bone marrow-derived cell mobilization and homing. Blood April 7, 2011 vol. 117 no. 14 3893-3902
    Species: Mouse

XV. Experiment Record Form

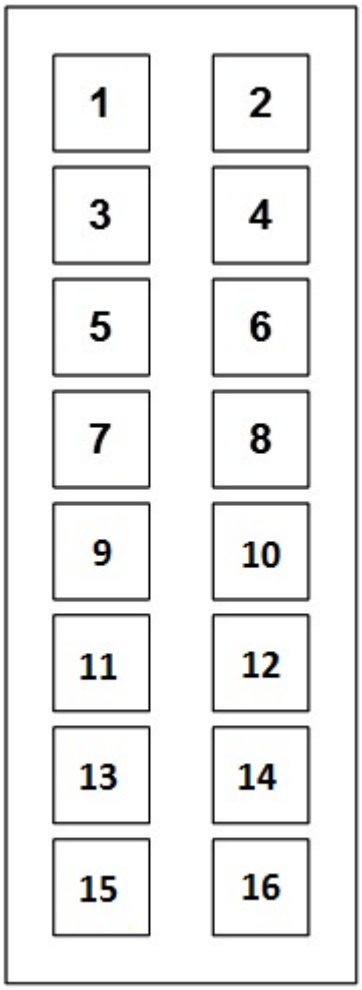
Date:_____

File Name:_____

Laser Power:_____

PMT:_____

Well No.	Sample Name	Dilution factor
1	CNTRL	
2	Std7	
3	Std6	
4	Std5	
5	Std4	
6	Std3	
7	Std2	
8	Std1	
9		
10		
11		
12		
13		
14		
15		
16		



XVI. How to Choose a Quantibody® Array?

Species-based selection:

Human (QAH-)	Mouse (QAM-)	Rat (QAR-)	Bovine (QAB-)	Canine (QAC-)
Equine (QAE-)	Feline (QAF-)	Primates (QAN-)	Porcine (QAP-)	Rabbit (QAL-)

Function-based selection:

Adhesion Molecule Arrays	Angiogenesis Arrays	Bone Metabolism Arrays	Chemokine Arrays
Custom Arrays	Cytokine Arrays	Growth Factor Arrays	IGF Signaling Arrays
IL-1 Family Arrays	Immune Response Arrays	Inflammation Arrays	Interleukin Arrays
Isotyping Arrays	MMP Arrays	Obesity Arrays	Ophthalmic Arrays
Periodontal Disease Arrays	Receptor Arrays	Th1/Th2/Th17 Arrays	

Cytokine Number-based selection:
Arrays are available in the Quantibody® platform to detect 1000 human, 640 mouse, or 67 rat proteins. GLP-Compliant testing services are also available.

To learn more about the Quantibody® Antibody Array, visit www.RayBiotech.com/Quantibody-Multiplex-Elisa-Array/