

# Quantibody<sup>®</sup> Human Chemokine Array 1

Quantitative measurement of 40 human cytokines

Catalog #: QAH-CHE-1

User Manual  
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Caution:  
Extraordinarily useful information enclosed



ISO 13485 Certified

3607 Parkway Lane, Suite 100  
Norcross, GA 30092

Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax: 770-206-2393  
Web: [www.RayBiotech.com](http://www.RayBiotech.com), Email: [info@raybiotech.com](mailto:info@raybiotech.com)

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**Please read the entire manual carefully before starting your experiment**

## I. Overview

<b>Cytokines Detected (40)</b>	6Ckine (CCL21), Axl, Betacellulin (BTC), CCL28 (MEC), CTACK (CCL27), CXCL16, ENA-78 (CXCL5), Eotaxin-3 (CCL26), GCP-2 (CXCL6), GRO, HCC-1 (CCL14), HCC-4 (CCL16), IL-9, IL-17F, IL-18 BP alpha, IL-28A, IL-29, IL-31, IP-10 (CXCL10), I-TAC (CXCL11), LIF, Light (TNFSF14), Lymphotactin (XCL1), MCP-2 (CCL8), MCP-3 (MARC/CCL7), MCP-4 (CCL13), MDC (CCL22), MIF, MIP-3 alpha (CCL20), MIP-3 beta (CCL19), MPIF-1 (CCL23), MSP alpha/beta, NAP-2 (PPBP/CXCL7), Osteopontin (SPP1), PARC (CCL18), Platelet Factor 4 (CXCL4), SDF-1 alpha (CXCL12 alpha), TARC (CCL17), TECK (CCL25), TSLP <i>See Section IX for Array Map</i>
<b>Format</b>	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
<b>Detection Method</b>	Fluorescence. Go to <a href="http://www.RayBiotech.com/Scanners">www.RayBiotech.com/Scanners</a> for a list of compatible laser scanners.
<b>Sample Volume</b>	50 - 100 µl per array
<b>Reproducibility</b>	CV <20%
<b>Assay Duration</b>	6 hours

## II. Introduction

Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in interactions between different cell types, cellular responses to environmental conditions, and maintenance of homeostasis. In addition, cytokines are also involved in most disease processes, including cancer and cardiac diseases.

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*.

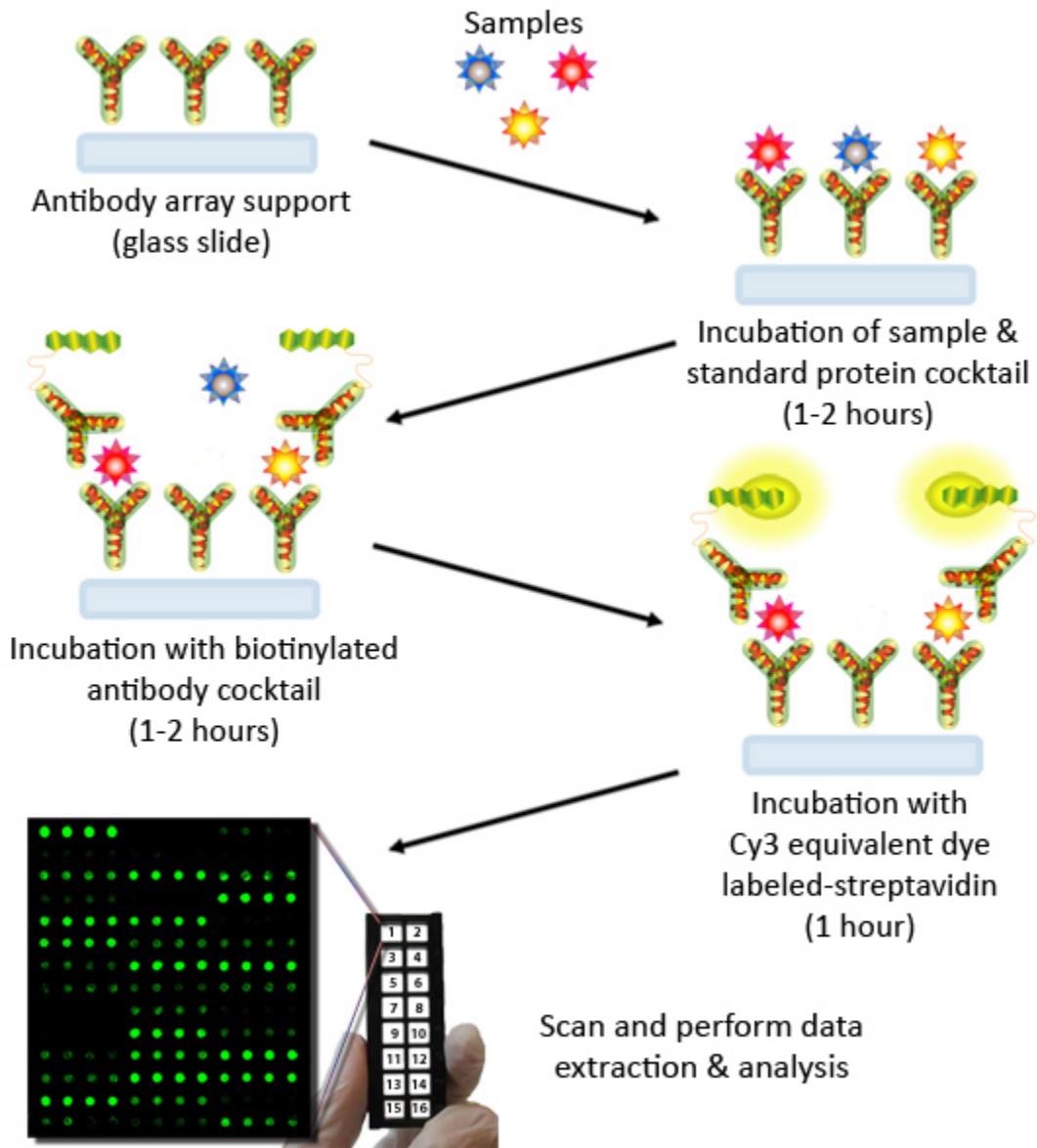
The Quantibody<sup>®</sup> array, our multiplexed sandwich ELISA-based quantitative array platform, enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity & specificity of ELISA and the high throughput 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<sup>®</sup> 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

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## IV. Materials Provided

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	QAH-CHE-1 S	Human Chemokine Array 1 Glass Slide	1	2
2	QA-SDB	Quantibody <sup>®</sup> 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-CHE-1 -STD	Human Chemokine Array 1 Lyophilized Standard Mix**	1 Vial	
6	QAH-CHE-1 B	Human Chemokine Array 1 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

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### 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  $\mu$ l 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  $\mu$ l, or 500  $\mu$ g lysate.
- The suggested serum/plasma dilution for this array is: 2x

### 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<sup>®</sup> 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  $\mu$ l 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

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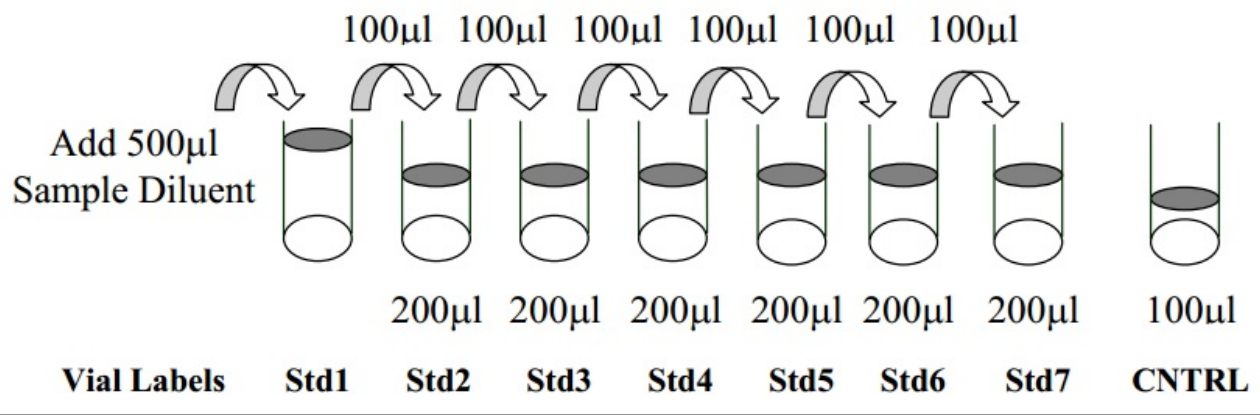
### A. Completely Air Dry The Glass Slide

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  $\mu$ l Sample Diluent to each of the tubes.
4. Pipette 100  $\mu$ l Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100  $\mu$ l Std2 to tube Std3 and so on.
5. Add 100  $\mu$ l 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  $\mu$ l Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.
7. Decant buffer from each well. Add 100  $\mu$ l 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  $\mu$ l of original or diluted serum, plasma, conditioned media, or other body fluid, or 250  $\mu$ g/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  $\mu$ l of sample or reagent is used.*

#### 8. Wash:

- Decant the samples from each well, and wash 5 times (5 min each) with 150  $\mu$ l 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 H<sub>2</sub>O.
- *(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

l), 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  $\mu$ l 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 H<sub>2</sub>O.

*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  $\mu$ l 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*

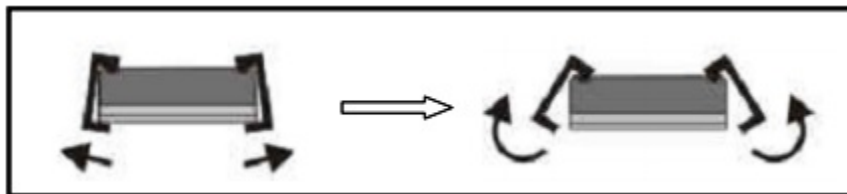
11. Decant the samples from each well, and wash 5 times (5 mins each) with 150  $\mu$ l of 1X Wash Buffer I and then 2 times with 150  $\mu$ l of 1x Wash Buffer II at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

#### **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  $\mu$ l 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.  
Decant the samples from each well, and wash 5 times (5 mins each) with 150  $\mu$ l of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step.
14.  $\mu$ l 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<sub>2</sub> stream.*

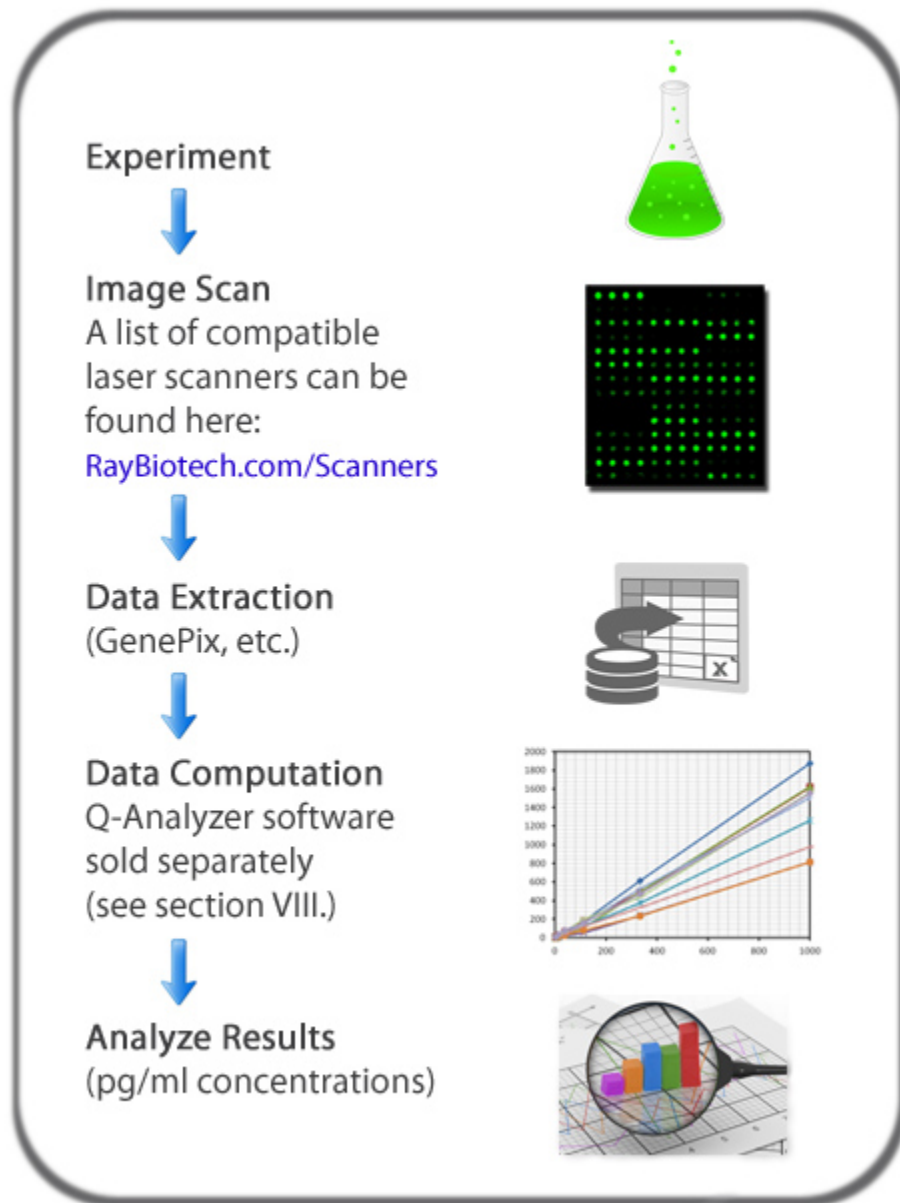
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains 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 along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). GAL files can be found here: [www.RayBiotech.com/Gal-Files.html](http://www.RayBiotech.com/Gal-Files.html).

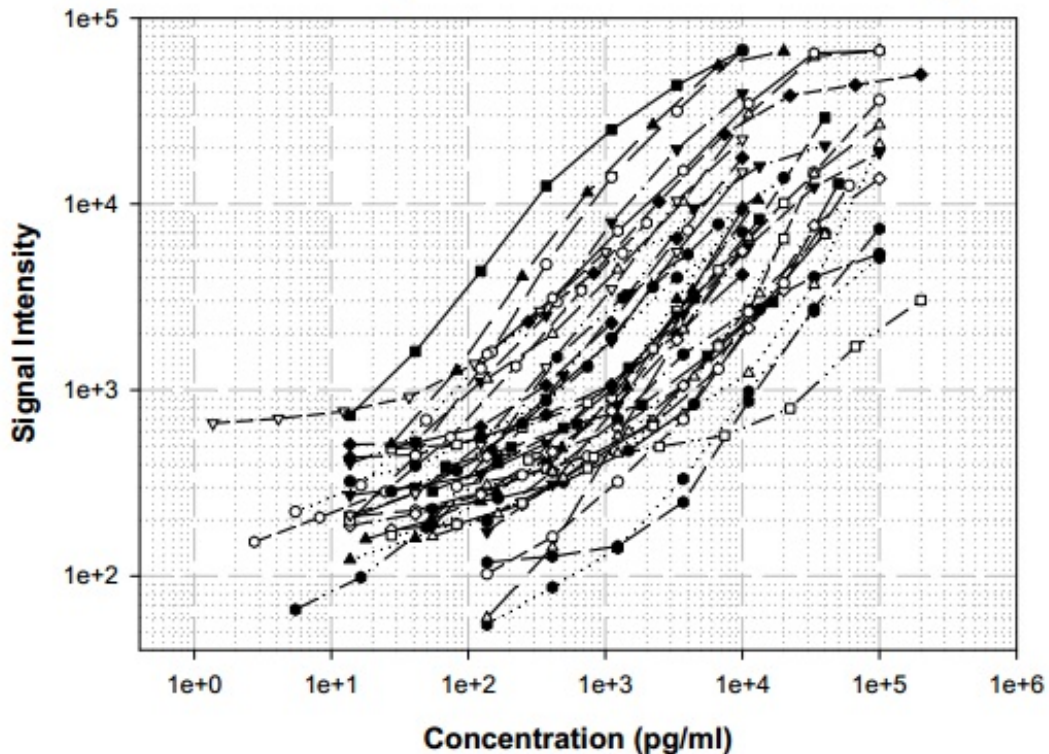
Need help analyzing all that data? Copy and paste your data into the Q-Analyzer Tool specific for this array, catalog number: **QAH-CHE-1-SW**. More information can be found in Section XII.



## IX. Array Map & Standard Curves

(hCHE-1 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				6Ckine (CCL21)			
B	Axl				Betacellulin (BTC)				CCL28 (MEC)			
C	CTACK (CCL27)				CXCL16				ENA-78 (CXCL5)			
D	Eotaxin-3 (CCL26)				GCP-2 (CXCL6)				GRO			
E	HCC-1 (CCL14)				HCC-4 (CCL16)				IL-9			
F	IL-17F				IL-18 BP alpha				IL-28A			
G	IL-29				IL-31				IP-10 (CXCL10)			
H	I-TAC (CXCL11)				LIF				LIGHT (TNFSF14)			
I	Lymphotactin				MCP-2 (CCL8)				MCP-3 (CCL7)			
J	MCP-4 (CCL13)				MDC (CCL22)				MIF			
K	MIP-3 alpha				MIP-3 beta				MPIF-1 (CCL23)			
L	MSP				NAP-2 (CXCL7)				Osteopontin (OPN)			
M	PARC (CCL18)				Platelet Factor 4 (PF4)				SDF-1 alpha			
N	TARC (CCL17)				TECK (CCL25)				TSLP			

**Quantibody Human Chemokine Array 1**



## X. Standard Concentrations

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

*Serial standard concentration (pg/ml)*

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
6Ckine	0	55	165	494	1,481	4,444	13,333	40,000
Axl	0	5	16	49	148	444	1,333	4,000
BTC	0	27	82	247	741	2,222	6,667	20,000
CCL28	0	55	165	494	1,481	4,444	13,333	40,000
CTACK	0	69	206	617	1,852	5,556	16,667	50,000
CXCL16	0	27	82	247	741	2,222	6,667	20,000
ENA-78	0	14	41	123	370	1,111	3,333	10,000
Eotaxin-3	0	27	82	247	741	2,222	6,667	20,000
GCP-2	0	14	41	123	370	1,111	3,333	10,000
GRO	0	1	4	12	37	111	333	1,000
HCC-1	0	5	16	49	148	444	1,333	4,000
HCC-4	0	14	41	123	370	1,111	3,333	10,000
IL-9	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-17F	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-18 BPa	0	82	247	741	2,222	6,667	20,000	60,000
IL-28A	0	14	41	123	370	1,111	3,333	10,000
IL-29	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-31	0	55	165	494	1,481	4,444	13,333	40,000
IP-10	0	14	41	123	370	1,111	3,333	10,000
I-TAC	0	14	41	123	370	1,111	3,333	10,000
LIF	0	18	53	160	481	1,444	4,333	13,000
LIGHT	0	14	41	123	370	1,111	3,333	10,000
Lymphotactin	0	137	412	1,235	3,704	11,111	33,333	100,000
MCP-2	0	3	8	25	74	222	667	2,000
MCP-3	0	5	16	49	148	444	1,333	4,000
MCP-4	0	14	41	123	370	1,111	3,333	10,000
MDC	0	14	41	123	370	1,111	3,333	10,000
MIF	0	5	16	49	148	444	1,333	4,000
MIP-3a	0	5	16	49	148	444	1,333	4,000
MIP-3b	0	27	82	247	741	2,222	6,667	20,000
MPIF-1	0	14	41	123	370	1,111	3,333	10,000
MSPa	0	137	412	1,235	3,704	11,111	33,333	100,000
NAP-2	0	5	16	49	148	444	1,333	4,000
OPN	0	137	412	1,235	3,704	11,111	33,333	100,000
PARC	0	5	16	49	148	444	1,333	4,000
PF4	0	137	412	1,235	3,704	11,111	33,333	100,000
SDF-1a	0	14	41	123	370	1,111	3,333	10,000
TARC	0	14	41	123	370	1,111	3,333	10,000
TECK	0	137	412	1,235	3,704	11,111	33,333	100,000
TSLP	0	14	41	123	370	1,111	3,333	10,000

## **XI. Spiking & Recovery**

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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.

*The spiking recovery rate for human culture media and serum*

ID	Spiking	H4522	H4522+Ag	H4522%	CM	CM+Ag	CM%
6Ckine	20,000	0	20,397	102.0%	0	24,169	120.8%
Axl	2,000	0	1,613	80.7%	0	2,282	114.1%
BTC	50,000	0	40,103	80.2%	0	45,084	90.2%
CCL28	20,000	0	2,559	12.8%	0	23,845	119.2%
CTACK	25,000	0	26,060	104.2%	0	27,022	108.1%
CXCL16	10,000	2,904	13,754	108.5%	0	12,661	126.6%
ENA-78	5,000	33	3,740	74.1%	0	3,244	64.9%
Eotaxin-3	10,000	0	10,415	104.2%	0	11,884	118.8%
GCP-2	5,000	0	1,053	21.1%	0	5,838	116.8%
GRO	500	0	385	77.1%	0	530	106.0%
HCC-1	2,000	6,628	5,107	-	0	2,127	106.3%
HCC-4	5,000	10,426	10,157	-	0	5,922	118.4%
IL-9	100,000	0	74,344	74.3%	0	111,508	111.5%
IL-17F	50,000	0	40,448	80.9%	0	35,992	72.0%
IL-18 BP <sub>a</sub>	30,000	0	23,375	77.9%	0	32,143	107.1%
IL-28A	5,000	0	4,301	86.0%	0	5,771	115.4%
IL-29	50,000	0	5,774	11.5%	0	48,391	96.8%
IL-31	20,000	0	14,447	72.2%	0	21,739	108.7%
IP-10	20,000	1,306	17,019	78.6%	0	20,767	103.8%
I-TAC	5,000	0	4,396	87.9%	0	3,742	74.8%
LIF	6,500	0	6,814	104.8%	0	7,795	119.9%
LIGHT	5,000	0	5,347	106.9%	0	5,382	107.6%
Lymphotactin	50,000	1,109	50,938	99.7%	0	48,343	96.7%
MCP-2	1,000	162	863	70.1%	0	888	88.8%
MCP-3	10,000	0	12,383	123.8%	0	11,669	116.7%
MCP-4	5,000	0	5,700	114.0%	0	5,173	103.5%
MDC	5,000	116	5,454	106.7%	0	5,023	100.5%
MIF	50,000	0	32,596	65.2%	0	42,962	85.9%
MIP-3 <sub>α</sub>	5,000	0	5,904	118.1%	0	3,435	68.7%
MIP-3 <sub>β</sub>	10,000	204	11,413	112.1%	0	11,541	115.4%
MPIF-1	200,000	0	226,293	113.1%	0	213,313	106.7%
MSP <sub>α</sub>	50,000	327,301	311,523	-	642	58,298	115.3%
NAP-2	10,000	8,572	11,963	33.9%	0	10,603	106.0%
OPN	50,000	1,536	56,526	110.0%	24,311	58,738	68.9%
PARC	5,000	5,947	6,293	6.9%	0	3,353	67.1%
PF4	50,000	97,015	89,464	-	0	56,628	113.3%
SDF-1 <sub>α</sub>	25,000	0	7,685	30.7%	0	27,502	110.0%
TARC	5,000	0	5,115	102.3%	0	5,795	115.9%
TECK	50,000	0	55,121	110.2%	0	48,732	97.5%
TSLP	20,000	0	16,657	83.3%	0	17,780	88.9%



## XII. Quantibody<sup>®</sup> Q-Analyzer

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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).

The Q-Analyzer Tool specific for this array is catalog number: **QAH-CHE-1-SW**.

### 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

<b>Problem</b>	<b>Cause</b>	<b>Recommendation</b>
<b>Weak Signal</b>	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.
<b>Uneven signal</b>	Bubble formed during incubation	Decrease amount of rocking during incubations. check for bubble formation and remove bubbles.
	Arrays are not completely covered by reagent	Completely cover arrays with solution for all required steps.
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
<b>Poor standard curve</b>	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.
<b>High background</b>	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. Publications Citing This Product

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1. Chowdhury UR., et al. Proteome Analysis of Human Aqueous Humor. Invest Ophthalmol Vis Sci. 2010 Oct;51(10):4921-31. doi: 10.1167/iovs.10-5531.  
**Species:** Human
2. Ratchford J., et al. A pilot study of functional electrical stimulation cycling in progressive multiple sclerosis. NeuroRehabilitation, Volume 27, Number 2 / 2010. 121-128DOI 10.3233/NRE-2010-0588  
**Species:** Human  
**Sample Type:** CSF
3. Chen YL., Huang KF., Kuo WC., et al. Inhibition of glutaminyl cyclase attenuates cell migration modulated by monocyte chemoattractant proteins. Biochem. J. (2012) 442, 403-412. doi:10.1042/BJ20110535  
**Species:** Human  
**Sample Type:** Conditioned Media
4. Laudanski P., Lemancewicz A., Kuc P., Charkiewicz K., et al. Chemokines Profiling of Patients with Preterm Birth. Mediators of Inflammation Volume 2014, Article ID 185758, 7 pages  
**Species:** Human  
**Sample Type:** Serum
5. Laudanski P., Zbucka-Kretowska M., Charkiewicz K., et al. Maternal Plasma and Amniotic Fluid Chemokines Screening in Fetal Down Syndrome. Mediators of Inflammation Volume 2014, Article ID 835837, 10 pages.  
<http://dx.doi.org/10.1155/2014/835837>  
**Species:** Human  
**Sample Type:** Plasma
6. Laudanski P., Zbucka-Kretowska M., Charkiewicz K., et al. Maternal Plasma and Amniotic Fluid Chemokines Screening in Fetal Down Syndrome. Mediators of Inflammation Volume 2014, Article ID 835837, 10 pages.  
<http://dx.doi.org/10.1155/2014/835837>  
**Species:** Human  
**Sample Type:** Other Fluid

*More citations for this product may be available.*

Contact [techsupport@raybiotech.com](mailto:techsupport@raybiotech.com).

# XV. Experiment Record Form

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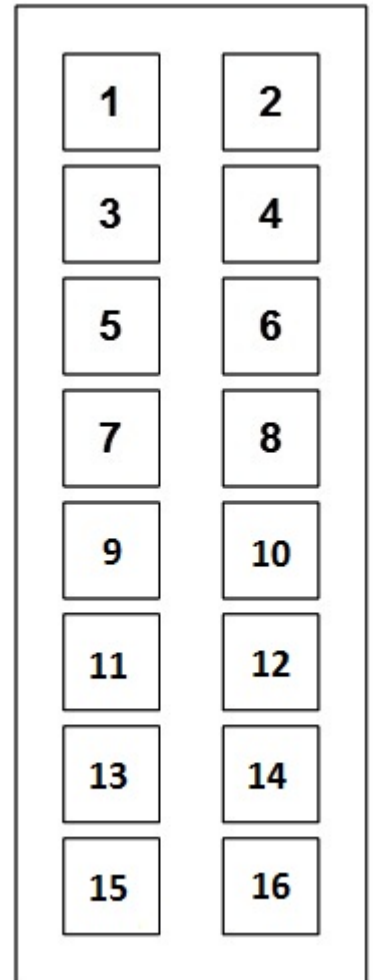
Date: \_\_\_\_\_

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<sup>®</sup> 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
<b>Custom Arrays</b>	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<sup>®</sup> platform to detect 1000 human, 200 mouse, or 67 rat proteins. GLP-Compliant testing services are also available.

To learn more about the Quantibody<sup>®</sup> Antibody Array, visit [www.RayBiotech.com/Quantibody-Multiplex-Elisa-Array.html](http://www.RayBiotech.com/Quantibody-Multiplex-Elisa-Array.html)

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