Quantibody® Mouse Cytokine Array 8

--Quantitative measurement of 40 Mouse cytokines

Patent Pending Technology

User Manual (Version Dec 2013)

Cat # QAM-CYT-8

RayBiotech, Inc.

We Provide You With Excellent
Protein Array Systems and Service

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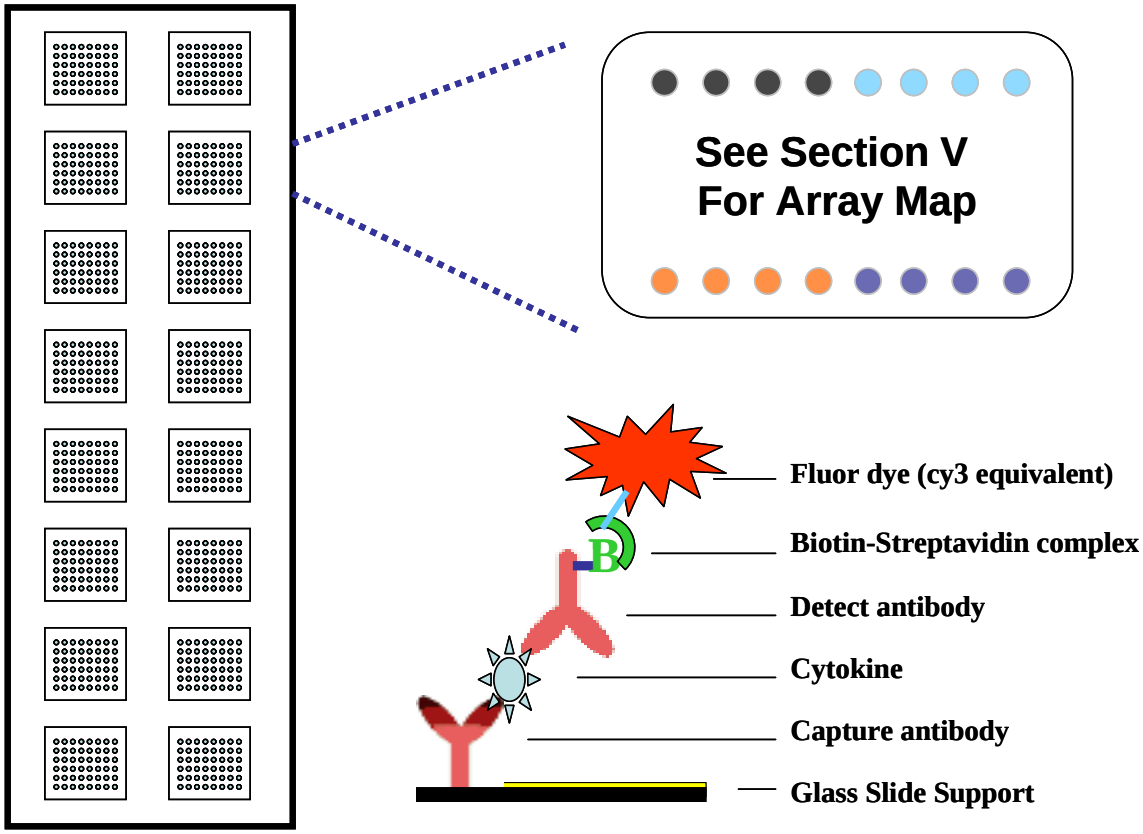
Cytokine Detected (40)	6Ckine, Activin A, ADAMTS1, Adiponectin, ANG-3, ANGPTL3, Artemin, CCL28, CD36, Chordin, CRP, E-Cadherin, Epigen, Epiregulin, Fas, Galectin-7, gp130, Granzyme B, Gremlin, IFN-γ R1, IL-17B, IL-17B R, IL-22, MIP-1β, MMP-2, MMP-3, MMP-10, PDGF-AA, Persephin, sFRP-3, Shh-N, SLAM, TCK-1, TECK, TGFβ1, TRANCE, TremL1, TWEAK, VEGF-B, VEGF-R2
Format	One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate.
Detection Method	Fluorescence with laser scanner: Cy3 equivalent dye
Sample Volume	50 – 100 µl per array
Reproducibility	CV <20%
Assay duration	6 hrs



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I. 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 array (ELISA). In this method, target protein is first 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 the traditional method works well for a single protein, the overall procedure is time consuming and requires a lot of sample. With little sample to work with, conservation of precious small quantities becomes a risky task. Take the advantage of advancement in microarray technology over the last decade; more and more choices are available to the scientist today. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which has now been widely applied in the research community with hundreds of peer reviewed publications such as in Cell and Nature.

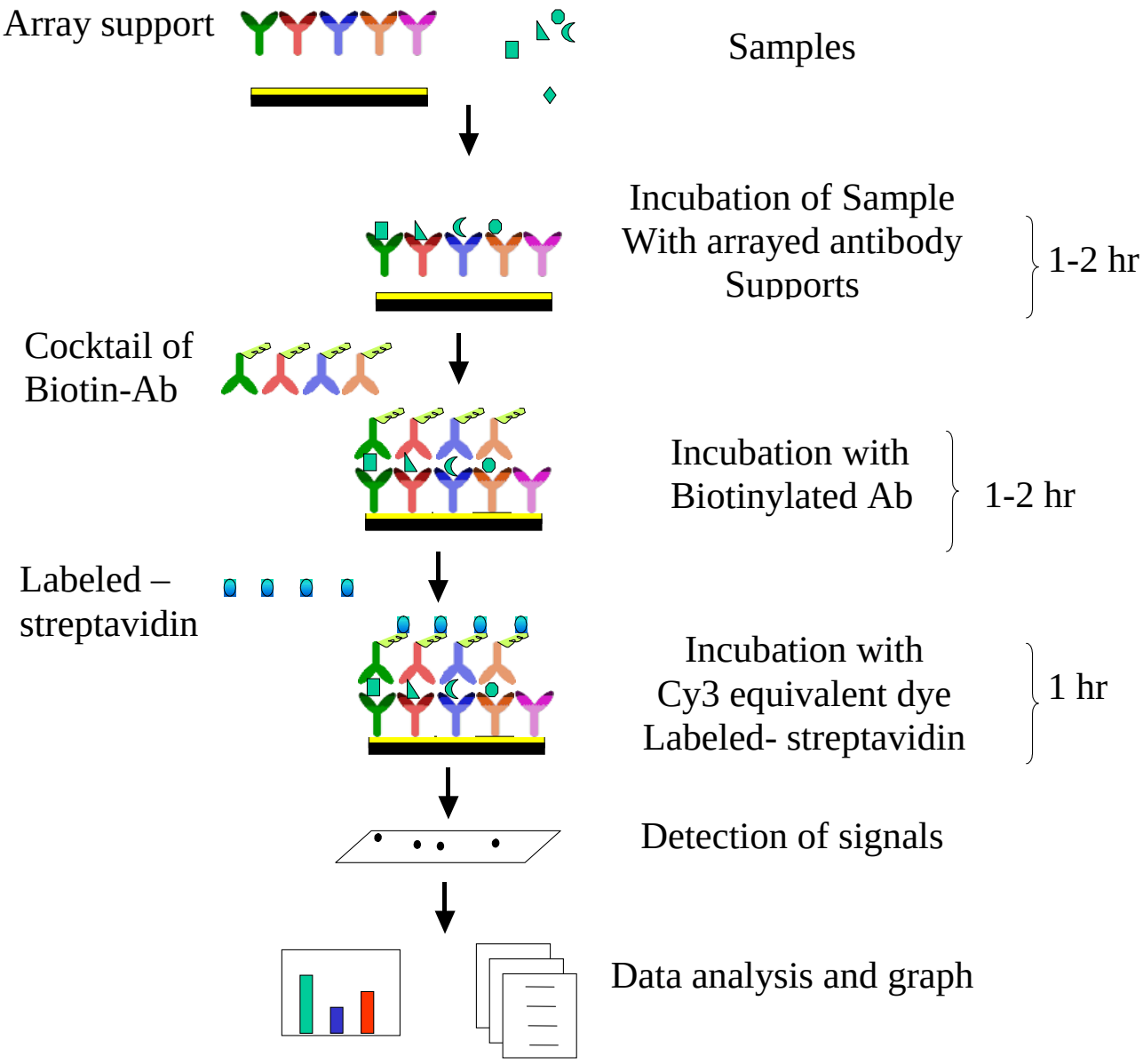
Quantibody® array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity / specificity of ELISA and the high throughput of the 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 isotope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled Cy3 equivalent dye using a laser scanner. Unlike the traditional ELISA, Quantibody products use array format. By arraying multiple cytokine

specific capture antibodies onto a glass support, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is spotted with 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 in one slide. Four slide chips can be nested into a tray, which matches a standard microplate 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 400 human, 200 mouse, or 100 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.

How It Works


Array support
Samples
Incubation of Sample
With arrayed antibody
Supports
1-2 hr
Cocktail of
Biotin-Ab
Incubation with
Biotinylated Ab
1-2 hr
Labeled –
streptavidin
Incubation with
Cy3 equivalent dye
Labeled- streptavidin
1 hr
Detection of signals
Data analysis and graph

II. Materials Provided

Upon receipt, all components of the Quantibody® Array kit should be stored at -20^{0}C. At -20^{0}C the kit will retain complete activity for up to 6 months. Once thawed, the glass chip, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at –20^{0}C and all other components may be stored at 4^{0}C. The entire kit should be used within 6 months of purchase.

Components

Item	Description	1-Slide kit	2-Slide kit
1	Quantibody® Array Glass Chip	1	2
2	Sample Diluent	1	1
3	20X Wash Buffer I	2	3
4	20X Wash Buffer II	1	1
5	Lyophilized cytokine standard mix *	1	1
6	Detection antibody cocktail	1	2
7	Cy3 equivalent dye-conjugated Streptavidin	1	2
8	Slide Washer/Dryer	1	1
9	Adhesive device sealer	5	10
10	Manual	1	1

** See Section VI for detailed cytokine concentrations after reconstitution.*

Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- 1.5ml Polypropylene microcentrifuge tubes

III. 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.
- We recommend the following parameters for your samples:
  50 to 100 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

  If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

B. Handling glass chips

- 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 latex free gloves.
- Handle glass chip in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, you might not have enough info to distinguish one slide from the other.

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 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 4^0C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

IV. Protocol

A. Completely air dry the glass chip

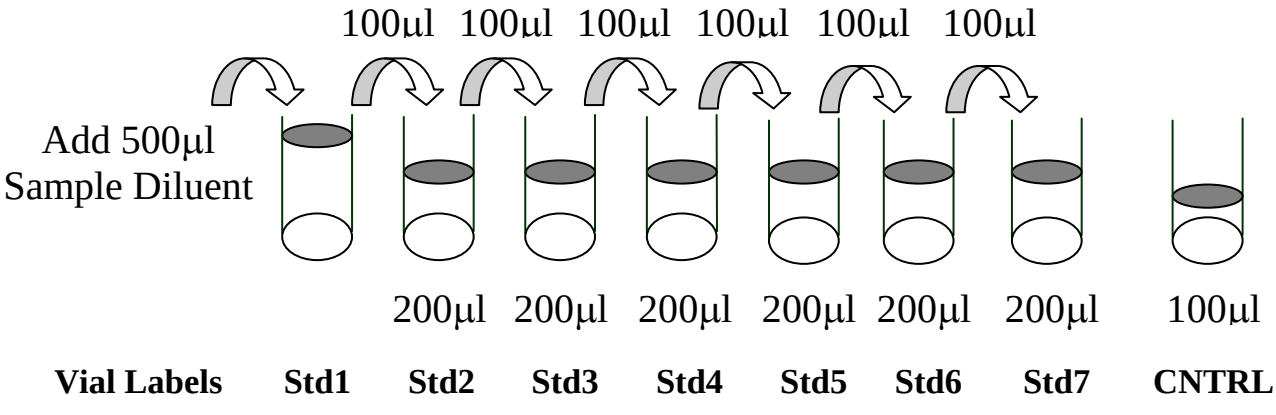
1. Take out the glass chip from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

Note: Incomplete drying of slides before use may cause the formation of "comet tails".

B. Prepare Cytokine Standard Dilutions

Note: 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 ^{0}C.

Prepare serial dilution of cytokine standards



2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500µl 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 200µl Sample Diluent to each of the tubes.

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

5. Add 100µl 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.

Note: 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 VI.

C. Blocking and Incubation

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

7. Decant buffer from each well. Add 100µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour. *(Longer incubation time is preferable for higher signals)*

Note: We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 ul of sample or reagent is used.

Note: This step may be done overnight at 4^0C for best results.

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 shaking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with H_2O.

- (*Optional for Cell and Tissue Lysates*) Put the glass chip 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 shaking 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 shaking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer II with H_2O.

Note: Incomplete removal of the wash buffer in each wash step may cause "dark spots". (Background signal is higher than that of the spot.)

D. Incubation with detection antibody cocktail and 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 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 shaking. Completely remove wash buffer in each wash step.

E. Incubation with Cy3 equivalent dye -Streptavidin and wash

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

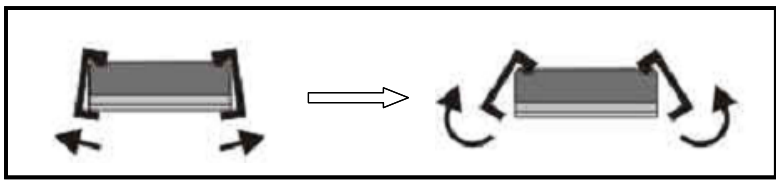
13. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.

14. Decant the samples from each well, and wash 5 times with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. 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) with gentle, and gently shake at room temperature for 5 minutes.

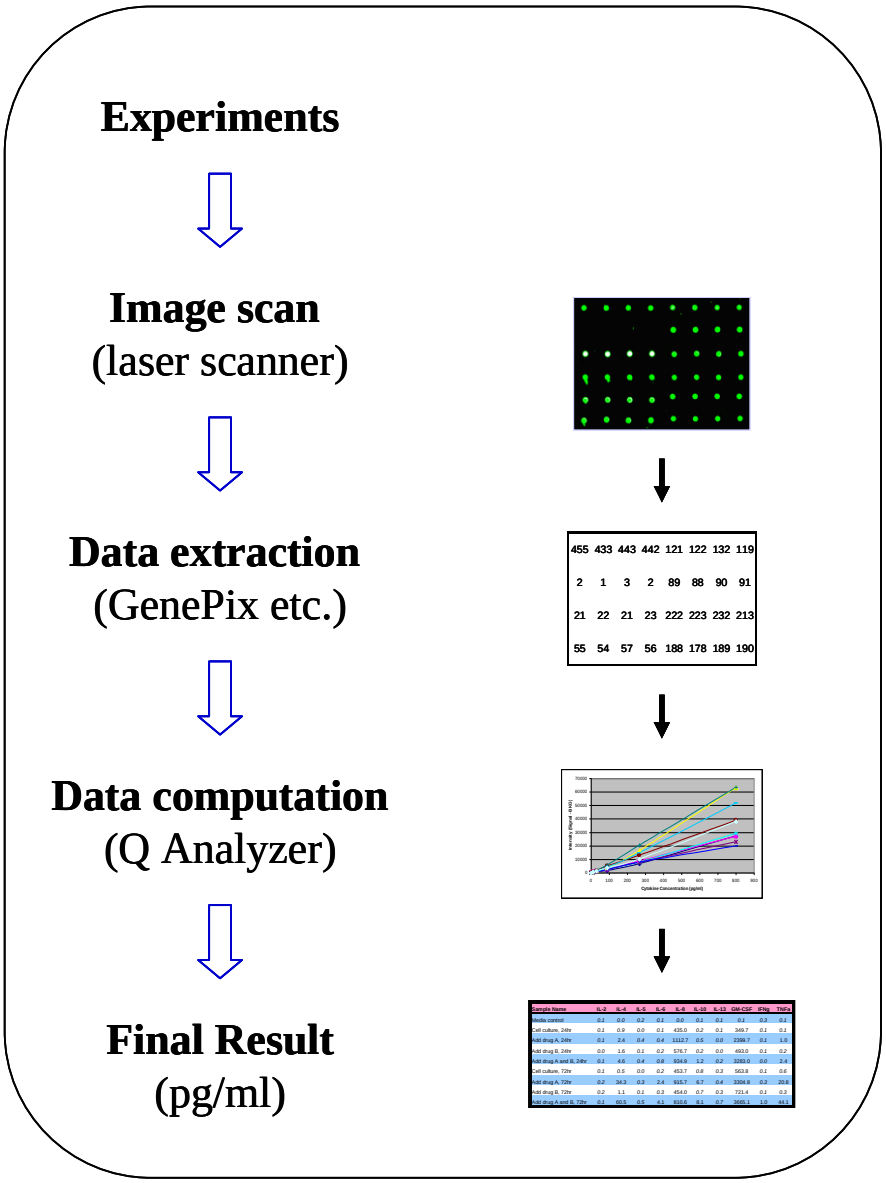
17. Remove water droplets completely by one of the following ways:
    - Put the glass chip into the Slide Washer/Dryer, and dry the glass chip by centrifuge at 1,000 rpm for 3 minutes without cap.
    - Or, dry the glass chip by a compressed N_2 stream.
    - Or gently apply suction with a pipette to remove water droplets. Do not touch the array, only the sides.

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

Note: 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

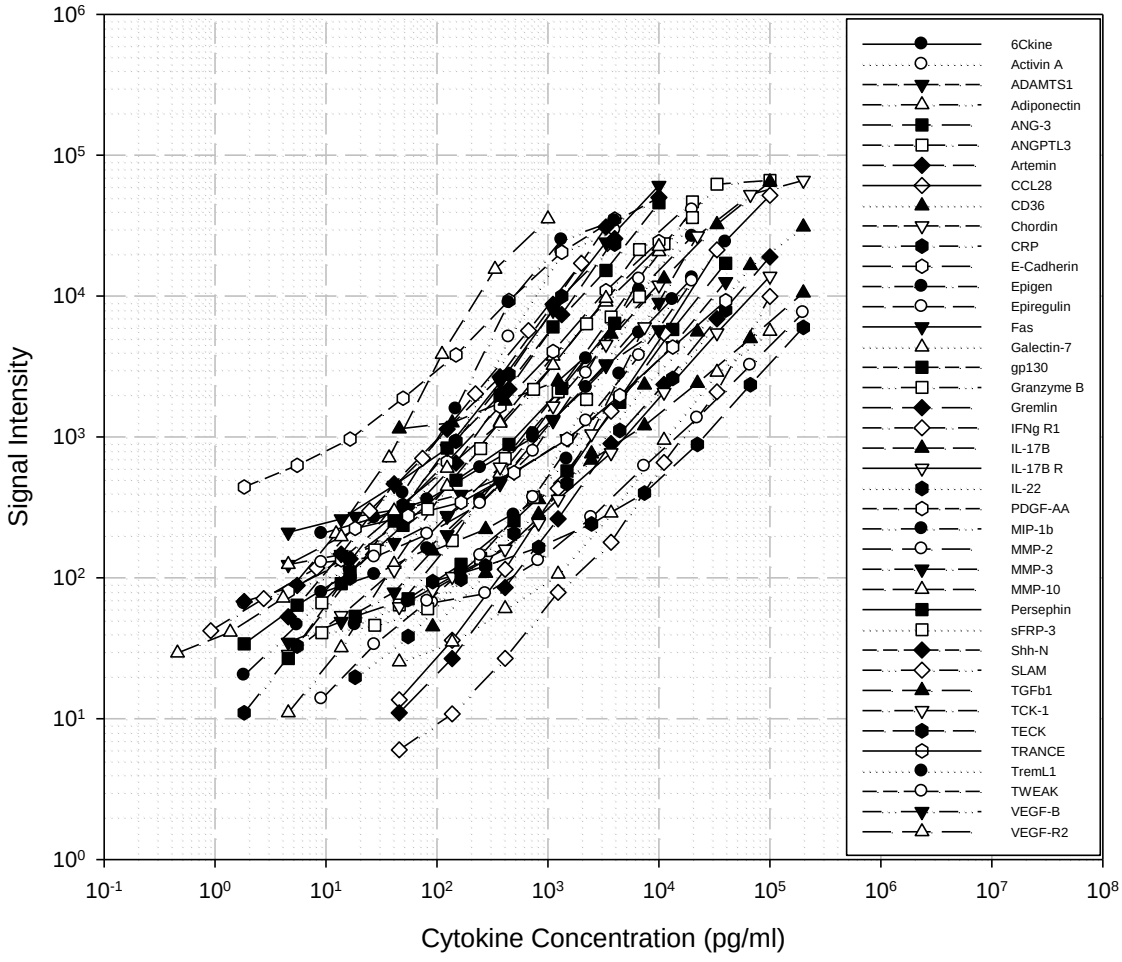
19. Data extraction can be done with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). For quantitative data analysis, our Quantibody® Q-Analyzer software is available. It gives visual output as well as digital values. More information can be found in section VIII.



V. Cytokine Array Map & Standard Curves

POS1	POS2	6Ckine
Activin A	ADAMTS1	Adiponectin
ANG-3	ANGPTL3	Artemin
CCL28	CD36	Chordin
CRP	E-Cadherin	Epigen
Epiregulin	Fas	Galectin-7
gp130	Granzyme B	Gremlin
IFN-γ R1	IL-17B	IL-17B R
IL-22	MIP-1β	MMP-2
MMP-3	MMP-10	PDGF-AA
Persephin	sFRP-3	Shh-N
SLAM	TCK-1	TECK
TGFβ1	TRANCE	TremL1
TWEAK	VEGF-B	VEGF-R2

QAM-CYT-8 Standard Curves



VI. 8-Point Standards

After reconstitution of the lyophilized cytokine standard mix, the 8-point cytokine concentration used for generating the standard curve of a given antigen is listed below. The detection sensitivity of each protein in one experiment is user dependent. Try our array specific Quantibody Q-Analyzer to see your Limit of Detection (LOD). (Section VIII).

Serial standard concentration (pg/ml)

(pg/ml)	Control	Std7	Std6	Std5	Std4	Std3	Std2	Std1
6Ckine	0	27	82	247	741	2,222	6,667	20,000
Activin A	0	5	16	49	148	444	1,333	4,000
ADAMTS1	0	55	165	494	1,481	4,444	13,333	40,000
Adiponectin	0	14	41	123	370	1,111	3,333	10,000
ANG-3	0	55	165	494	1,481	4,444	13,333	40,000
ANGPTL3	0	137	412	1,235	3,704	11,111	33,333	100,000
Artemin	0	5	16	49	148	444	1,333	4,000
CCL28	0	137	412	1,235	3,704	11,111	33,333	100,000
CD36	0	274	823	2,469	7,407	22,222	66,667	200,000
Chordin	0	14	41	123	370	1,111	3,333	10,000
CRP	0	5	16	49	148	444	1,333	4,000
E-Cadherin	0	14	41	123	370	1,111	3,333	10,000
Epigen	0	27	82	247	741	2,222	6,667	20,000
Epiregulin	0	274	823	2,469	7,407	22,222	66,667	200,000
Fas	0	14	41	123	370	1,111	3,333	10,000
Galectin-7	0	137	412	1,235	3,704	11,111	33,333	100,000
gp130	0	14	41	123	370	1,111	3,333	10,000
Granzyme B	0	27	82	247	741	2,222	6,667	20,000
Gremlin	0	137	412	1,235	3,704	11,111	33,333	100,000
IFNγ R1	0	3	8	25	74	222	667	2,000
IL-17B	0	274	823	2,469	7,407	22,222	66,667	200,000
IL-17B R	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-22	0	55	165	494	1,481	4,444	13,333	40,000
MIP-1β	0	5	16	49	148	444	1,333	4,000
MMP-2	0	27	82	247	741	2,222	6,667	20,000
MMP-3	0	14	41	123	370	1,111	3,333	10,000
MMP-10	0	1	4	12	37	111	333	1,000
PDGF-AA	0	5	16	49	148	444	1,333	4,000
Persephin	0	5	16	49	148	444	1,333	4,000
sFRP-3	0	27	82	247	741	2,222	6,667	20,000
Shh-N	0	14	41	123	370	1,111	3,333	10,000
SLAM	0	137	412	1,235	3,704	11,111	33,333	100,000
TCK-1	0	274	823	2,469	7,407	22,222	66,667	200,000
TECK	0	274	823	2,469	7,407	22,222	66,667	200,000
TGFβ1	0	137	412	1,235	3,704	11,111	33,333	100,000
TRANCE	0	55	165	494	1,481	4,444	13,333	40,000
TremL1	0	55	165	494	1,481	4,444	13,333	40,000
TWEAK	0	27	82	247	741	2,222	6,667	20,000
VEGF-B	0	14	41	123	370	1,111	3,333	10,000
VEGF-R2	0	14	41	123	370	1,111	3,333	10,000

VII. System Recovery

The antibody pairs used in the kit have been tested to recognize their specific antigen. The spiking recovery rate of the cytokines by the kit in 2x diluted Mouse serum and 2x diluted Mouse cell culture media (CM) is listed in the following table.

The spiking recovery rate for Mouse culture media and serum

(pg/ml)	Spiking	Serum	Serum+Ag	Serum%	CM	CM+Ag	CM%
6Ckine	10,000	538	8,565	80%	121	13,298	132%
Activin A	2,000	67	1,934	93%	6	2,139	107%
ADAMTS1	20,000	1,122	21,547	102%	199	32,973	164%
Adiponectin	5,000	3,184	8,267	102%	37	5,842	116%
ANG-3	20,000	101	16,257	81%	302	37,195	184%
ANGPTL3	50,000	33,671	80,523	94%	96	69,881	140%
Artemin	2,000	4,900	4,623	-	15	2,560	127%
CCL28	50,000	3,525	90,578	174%	779	70,006	138%
CD36	100,000	2,351	69,777	67%	381	104,701	104%
Chordin	5,000	423	3,283	57%	86	4,747	93%
CRP	2,000	2,655	3,143	24%	12	1,021	50%
E-Cadherin	5,000	27,057	25,069	-	39	4,876	97%
Epigen	10,000	175	11,309	111%	71	11,468	114%
Epiregulin	100,000	3,159	90,117	87%	3,683	157,198	154%
Fas	5,000	383	6,105	114%	45	6,192	123%
Galectin-7	50,000	1,552	28,097	53%	886	65,013	128%
gp130	5,000	551	3,548	60%	20	4,344	86%
Granzyme B	10,000	1,365	8,990	76%	88	9,218	91%
Gremlin	50,000	652	45,951	91%	1,179	74,555	147%
IFNγ R1	1,000	5	838	83%	7	1,156	115%
IL-17B	100,000	12,840	113,155	100%	3,836	177,202	173%
IL-17B R	50,000	1,142	34,580	67%	997	63,888	126%
IL-22	20,000	1,105	21,325	101%	693	39,788	195%
MIP-1β	2,000	13	1,053	52%	12	1,181	58%
MMP-2	10,000	2,408	5,116	27%	49	5,418	54%
MMP-3	5,000	4,590	8,969	88%	190	11,440	225%
MMP-10	500	1,454	1,445	-	2	585	117%
PDGF-AA	2,000	1,906	3,910	100%	140	2,510	118%
Persephin	2,000	71	1,247	59%	65	2,976	146%
sFRP-3	10,000	377	5,213	48%	4,713	13,057	83%
Shh-N	5,000	23	6,044	120%	1,569	5,908	87%
SLAM	50,000	1,086	49,132	96%	839	101,884	202%
TCK-1	100,000	176,932	163,930	-	358	82,380	82%
TECK	200,000	11,010	183,351	86%	4,656	192,414	94%
TGFβ1	50,000	512	31,842	63%	594	64,434	128%
TRANCE	20,000	4,047	20,367	82%	336	28,533	141%
TremL1	20,000	40,476	52,629	61%	330	32,191	159%
TWEAK	20,000	232	16,917	83%	1,876	23,365	107%
VEGF-B	5,000	173	3,798	73%	136	9,077	179%
VEGF-R2	5,000	94	2,456	47%	24	3,577	71%

VIII. Quantibody® Q-Analyzer

Quantibody Q-Analyzer is an array specific, Excel-based program. However, it is not a simple calculation macro as it contains sophisticated data analysis.

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 number of samples.
- *Two Positive Controls*: The program takes 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 those 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.

IX. 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	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Don't make too low dilution or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed covered by reagent	Completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer
	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 that 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 laser power
	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.

X. Select Quantibody Publications

Quantibody products have been referenced in hundreds of publications. Following are only some of the selections.

- Gao, et al. A Chinese herbal decoction, Danggui Buxue Tang, activates extracellular signal-regulated kinase in cultured T-lymphocytes. *FEBS Letters*, 2007; 581(26): 5087-5093.
- Zhai, et al. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. *Molecular and Cellular Biology*. 2008; 28(24): 7414-7426.
- Toh HC et al: Clinical Benefit of Allogeneic Melanoma Cell Lysate–Pulsed Autologous Dendritic Cell Vaccine in MAGE-Positive Colorectal Cancer Patients. *Clin Cancer Res* 2009 15:7726-7736
- Willingham, SB et al. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol*. 2009; 183(3):2008-15
- Gujar SA et al: Reovirus virotherapy overrides tumor antigen presentation evasion and promotes protective antitumor immunity. *Mol Cancer Ther*. 2010 9(11):2924-33
- Rajkumar T et al: Identification and validation of genes involved in gastric tumorigenesis. *Cancer Cell Int*. 2010 10(1):45
- Lewis JA et al: Analysis of Secreted Proteins as an in vitro Model for Discovery of Liver Toxicity Markers. *J Proteome Res*. 2010; 9(11):5794-802
- Montes AH et al: The MMP1 (-16071G/2G) single nucleotide polymorphism associates with the HAART-related lipodystrophic syndrome. *AIDS*. 2010 24(16):2499-506
- Yin JJ et al: Cediranib/AZD2171 Inhibits Bone and Brain Metastasis in a Preclinical Model of Advanced Prostate Cancer. *Cancer Res*. 2010 70(21):8662-73
- Storti, P et al: HOXB7 expression by myeloma cells regulates their pro-angiogenic properties in multiple myeloma patients. *Leukemia*, 2011 Mar;25(3):527-37
- Shinmura D et al: Pretreatment of Human Mesenchymal Stem Cells with Pioglitazone Improved Efficiency of Cardiomyogenic Transdifferentiation and Improved Cardiac Function. *Stem Cells*. 2011 Feb;29(2):357-66.
- Feng W. et al: A novel role for platelet secretion in angiogenesis: mediating bone marrow-derived cell mobilization and homing. *Blood.* 2011 Mar 2.
- Fleming J. et al: Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. *Mult Scler*. 2011 Mar 3.
- Morelli T et al: Angiogenic biomarkers and healing of living cellular constructs. *J Dent Res*. 2011 Apr;90(4):456-62.
- Christoph E. et al: Levels of VEGF but not VEGF165b are Increased in the Vitreous of Patients With Retinal Vein Occlusion. *Am J of Ophthalmology* 2011. May 28
- Lee A et al: Bacterial and Salivary Biomarkers Predict the Gingival Inflammatory Profile. *J Periodontol*. 2011 May 12.
- Scanlon ST, et al: Airborne lipid antigens mobilize resident intravascular NKT cells to induce allergic airway inflammation. *J Exp Med*. 2011 Sep 19.

XI. Experiment Record Form

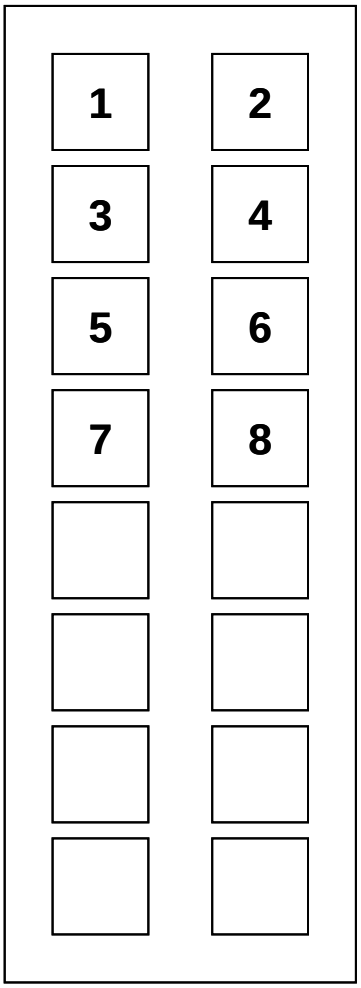
Date: _____

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		



XII. How to Choose Quantibody® Products?

Species-based selection:

- **Human**: (QAH-)
- **Mouse**: (QAM-)
- **Rat**: QAR
- **Porcine**: QAP-CYT-1
- **Non-Human Primates (NHP)**: QAN-CYT-1
- **Canine**: QAC-CYT-1
- **Feline**: QAF-CYT-1
- **Equine**: QAE-CYT-1
- **Bovine**: QAB-CYT-1

Function-based selection:

- **TH1/TH2/TH17 Array:** QAH-TH-1, QAH-TH17, QAM-TH17
- **Inflammation Arrays:** QAH-INF-1, QAH-INF-2, QAH-INF-3; QAM-INF-1; QAR-INF-1
- **Angiogenesis Arrays:** QAH-ANG-1, QAH-ANG-2, QAH-ANG-3, QAH-ANG-1000
- **Chemokine Arrays:** QAH-CHE-1, QAM-CHE-1
- **MMP Array:** QAH-MMP-1
- **Immunoglobin Isotype Array:** QAH-ISO-1; AAM-ISO-G1
- **Dried Eye Disease**: QAH-DED-1
- **Periodontal Disease**: QAH-PDD-1
- **Sepsis Biomarker**: QAH-SEP-1

Cytokine Number-based selection:

- **360 cytokines:** QAH-CAA-8000
- **320 cytokines:** QAH-CAA-7000
- **280 cytokines:** QAH-CAA-6000
- **240 cytokines:** QAH-CAA-5000
- **200 cytokines:** QAH-CAA-4000; QAM-CAA-4000
- **160 cytokines:** QAH-CAA-3000, QAM-CAA-3000
- **120 cytokines:** QAH-CAA-2000; QAM-CAA-2000
- **80 cytokines:** QAH-CAA-1000; QAM-CAA-1000
- **60 cytokines:** QAH-ANG-1000; QAM-CYT-Q2000
- **40 cytokines:** QAH-INF-3, QAH-CHE-1, QAH-GF-1, QAH-REC-1, QAH-CYT-4,
- **30 cytokines:** QAH-ANG-2, QAH-ANG-3, QAM-INT-1000, QAR-CYT-3, QAM-CHE-1
- **20 cytokines:** QAH-CYT-1, QAH-CYT-2, QAM-CYT-1, QAM-CYT-2, QAM-CYT-3
- **10 cytokines:** QAH-TH-1, QAH-INF-1, QAH-INF-2, QAH-ANG-1, QAH-MMP-1
- **less than 10 cytokines:** QAH-ISO-1, QAH-ADI-2, QAP-CYT-1, QAM-ISO-G1

Purpose-based selection --- Custom Arrays

- Choose from over 1000 cytokine pool; Any kind; Any number
- Order slide only or full service in house
- Desired marker not in our pool? No problem! For certain developmental fee, we may be able to add the marker to your panel if the paired antibodies are available on the market.

Note:

Quantibody® is the trademark of RayBiotech, Inc.

Cytokine protein arrays are RayBiotech patent-pending technology.

This product is intended for research only and is not to be used for clinical diagnosis. Our produces may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

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Products are guaranteed for three months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

This product is for research use only.