Quantibody® Human Cytokine Antibody Array X00

A combination of 25 non-overlapping arrays to quantitatively measure 1000 human cytokines

Catalog #: QAH-CAA-X00

User Manual
Last revised July 18, 2019

Caution:
Extraordinarily useful information enclosed

RayBiotech
Empowering your proteomics

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, Email: info@raybiotech.com

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Please read the entire manual carefully before starting your experiment

I. Overview

Cytokines Detected (1000)	Arrays Included: QAH-INF-3 (40); QAH-GF-1 (40); QAH-CHE-1 (40); QAH-REC-1 (40); QAH-CYT-4 (40); QAH-CYT-5 (40); QAH-CYT-6 (40); QAH-CYT-7 (40); QAH-CYT-8 (40); QAH-CYT-9 (40); QAH-CYT-10 (40); QAH-CYT-11 (40); QAH-CYT-12 (40); QAH-CYT-13 (40); QAH-CYT-14 (40); QAH-CYT-15 (40); QAH-CYT-16 (40); QAH-CYT-17 (40); QAH-CYT-18 (40); QAH-CYT-19 (40); QAH-CYT-20 (40); QAH-CYT-21 (40); QAH-CYT-22 (40); QAH-CYT-23 (40); QAH-CYT-24 (40) *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

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

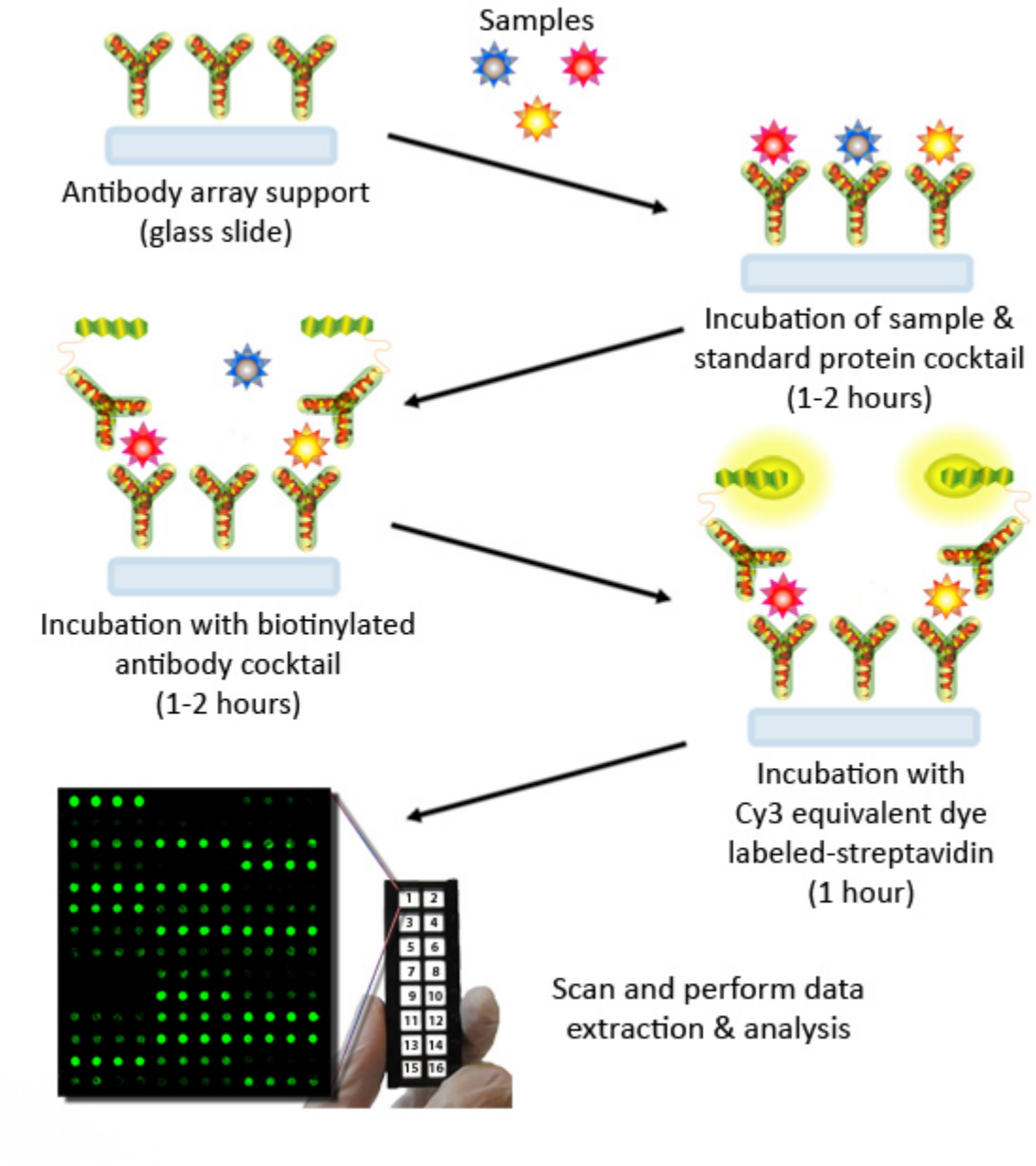
this problem. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which have now been widely applied in the research community with hundreds of peer reviewed publications, including top-tier journals such as *Cell* and *Nature*.

The Quantibody® 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

This product is a combination of multiple arrays. Items 1, 5, & 6 are array-specific.

	Catalog #	Component Name	1 Slide Box	2 Slide Box*
1	*[Array-Cat-#]* S	*Array-specific* 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	*[Array-Cat-#]* -STD	*Array-specific* Lyophilized Standard Mix**	1 Vial	
6	*[Array-Cat-#]* B	*Array-specific* 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: 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® 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

Note: *This product contains sets of reagents for different arrays. Always ensure you are using the proper glass slide, lyophilized standard mix, and biotinylated antibody cocktail for the correct corresponding array.*
The following procedure is for processing any one of the arrays in the kit.

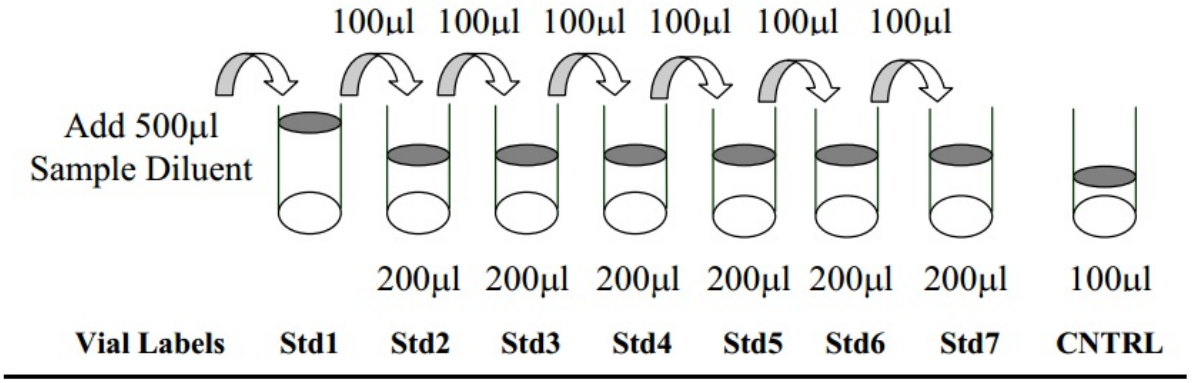
A. Completely Air Dry The Glass Slide

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

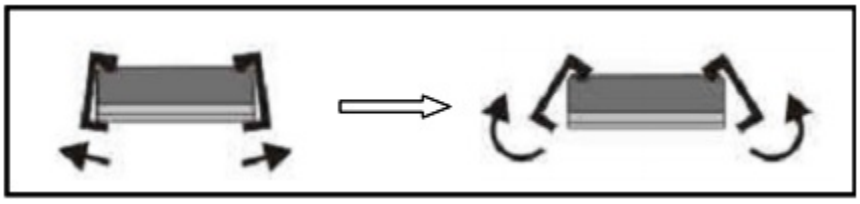
12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

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.

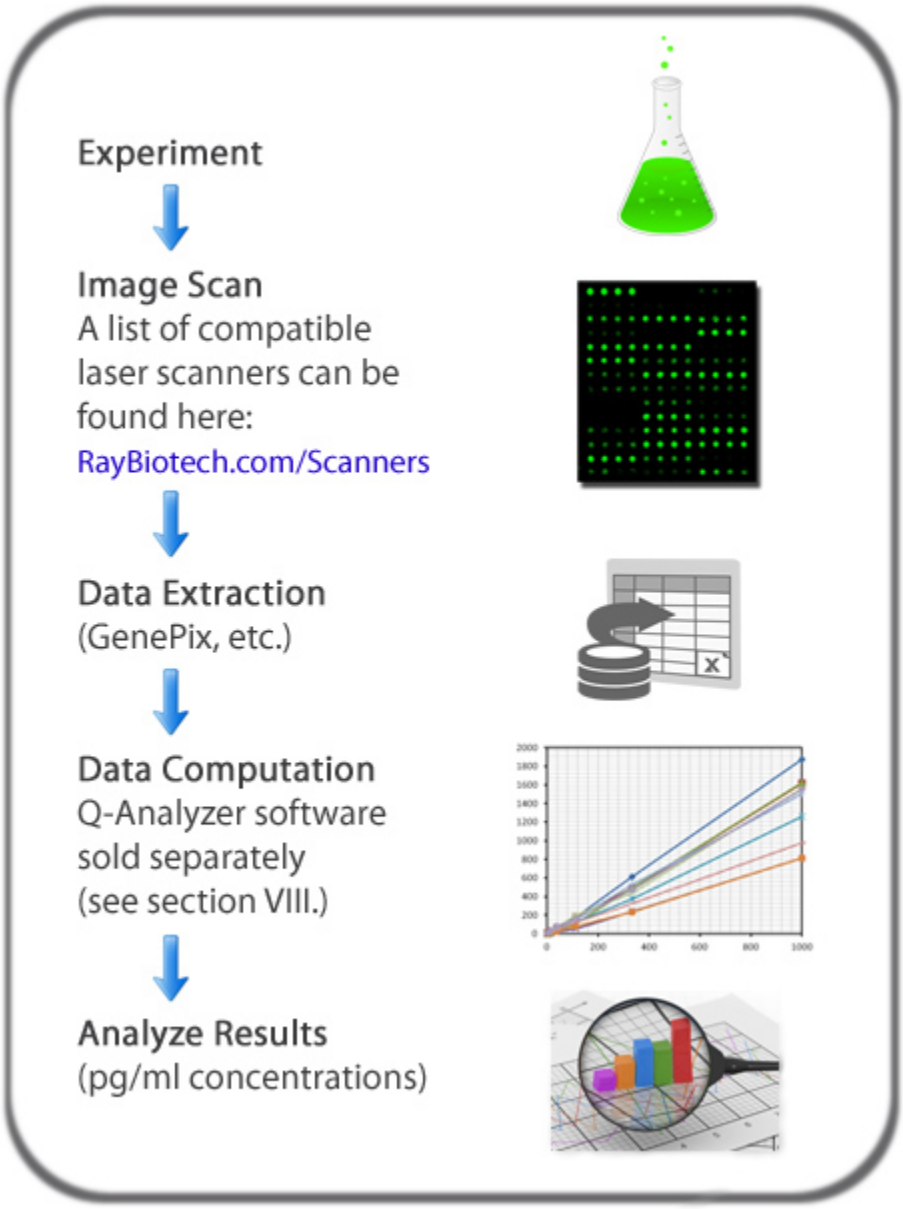
18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix or Innopsys Innoscan. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains 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 along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.). GAL files can be found here: 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-CAA-X00-SW**. More information can be found in Section XII.



IX. Array Map & Standard Curves

Please view the individual array manuals for representative standard curve images

QAH-INF-3

(hINF-3 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				BLC (CXCL13)			
B	Eotaxin-1 (CCL11)				Eotaxin-2 (MPIF-2)				G-CSF			
C	GM-CSF				I-309				ICAM-1 (CD54)			
D	IFN gamma				IL-1 alpha				IL-1 beta			
E	IL-1ra (IL-1 F3)				IL-2				IL-4			
F	IL-5				IL-6				IL-6sR			
G	IL-7				IL-8				IL-10			
H	IL-11				IL-12p40				IL-12p70			
I	IL-13				IL-15				IL-16			
J	IL-17				MCP-1 (CCL2)				M-CSF			
K	MIG (CXCL9)				MIP-1 alpha (CCL3)				MIP-1 beta (CCL4)			
L	MIP-1 delta (CCL15)				PDGF-BB				RANTES (CCL5)			
M	TIMP-1				TIMP-2				TNF alpha			
N	TNF beta				TNF RI				TNF RII			

QAH-GF-1

(hGF-1 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AR			
B	BDNF				bFGF				BMP-4			
C	BMP-5				BMP-7				beta-NGF			
D	EGF				EGF R				EG-VEGF			
E	FGF-4				FGF-7 (KGF)				GDF-15			
F	GDNF				Growth Hormone (GH)				HB-EGF			
G	HGF				IGFBP-1				IGFBP-2			
H	IGFBP-3				IGFBP-4				IGFBP-6			
I	IGF-I				Insulin				MCF R			
J	NGFR (TNFSR16)				NT-3				NT-4			
K	Osteoprotegerin (OPG)				PDGF-AA				PlGF (PLGF)			
L	SCF				SCF R (CD117)				TGF alpha			
M	TGF beta 1				TGF beta 3				VEGF-A (VEGF)			
N	VEGF R2				VEGF R3				VEGF-D			

QAH-CHE-1

(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			

QAH-REC-1

(hREC-1 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				4-1BB (CD137)			
B	ALCAM (CD166)				B7-1 (CD80)				BCMA (TNFRSF17)			
C	CD14				CD30 (TNFRSF8)				CD40 Ligand			
D	CEACAM-1				DR6 (TNFRSF21)				Dtk			
E	Endoglin (CD105)				ErbB3				E-Selectin			
F	Fas				Flt-3 Ligand				GITR (TNFRSF18)			
G	HVEM (TNFRSF14)				ICAM-3 (CD50)				Contactin-2			
H	IL-1 RI				IL-2 R gamma				IL-10 R beta			
I	IL-17R				IL-21 R				LIMPII			
J	Lipocalin-2 (NGAL)				L-Selectin (CD62L)				LYVE-1			
K	MICA				MICB				NRG1-beta 1			
L	PDGF R beta				PECAM-1 (CD31)				RAGE			
M	TIM-1 (KIM-1)				TRAIL R3				Trappin-2			
N	uPAR				VCAM-1				XEDAR			

QAH-CYT-4

(hCYT-4 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin A			
B	AgRP				Angiogenin (ANG)				Angiopoietin-1 (ANG-1)			
C	Angiostatin				Catheprin S				CD40			
D	Cripto-1				DAN				DKK-1			
E	E-Cadherin				EpCAM (TROP1)				Fas Ligand (TNFSF6)			
F	Fc gamma RIIB/C				Follistatin				Galectin-7			
G	ICAM-2 (CD102)				IL-13 R1				IL-13 R alpha 2			
H	IL-17B				IL-2 R alpha				IL-2 R beta			
I	IL-23				LAP/TGF beta 1				NrCAM			
J	PAI-I				PDGF-AB				Resistin			
K	SDF-1 beta				sgp130				Shh N			
L	Siglec-5 (CD170)				ST2 (IL-1 R4)				TGF-beta 2			
M	Tie-2				Thrombopoietin (TPO)				TRAIL-R4			
N	TREM-1				VEGF-C				VEGF-R1			

QAH-CYT-5

(hCYT-5 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Adiponectin (ACRP30)			
B	Adipsin				Alpha-fetoprotein (AFP)				ANGPTL4			
C	Beta-2 Microglobuliin (B2M				BCAM				CA125			
D	CA15-3				CEA				CRP			
E	ErbB2				Ferritin				FSH			
F	GRO alpha (CXCL1)				HCG beta (HCGb)				IGF-I R			
G	IL-1 RII				IL-3				IL-18 R beta			
H	IL-21				Leptin				MMP-1			
I	MMP-2				MMP-3				MMP-8			
J	MMP-9				MMP-10				MMP-13			
K	NCAM-1 (CD56)				Nidogen-1				NSE			
L	Oncostatin M (OSM)				Procalcitonin (PCT)				Prolactin			
M	PSA-free				Siglec-9				TACE			
N	Thyroglobulin				TIMP-4				TSH			

QAH-CYT-6

(hCYT-6 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				2B4 (CD244)			
B	ADAM-9				Angiopoietin-2 (ANG-2)				APRIL			
C	BMP-2				BMP-9				C5a			
D	Cathepsin L				CD200				CD97			
E	Chemerin				DcR3				FABP2			
F	FAP				FGF-19				Galectin-3			
G	HGF R				IFN alpha/beta R2				IGF-II			
H	IGF-II R				IL-1 R6 (IL-1 Rrp2)				IL-24			
I	IL-33 (IL-1 F11)				Kallikrein 14				Legumain			
J	LOX-1				MBL				Neprilysin			
K	Notch-1				NOV (CCN3)				Osteoactivin			
L	PD-1				PGRPs				Serpin A4			
M	sFRP-3				Thrombomodulin				TLR2			
N	TRAIL R1				Transferrin				WIF-1			

QAH-CYT-7

(hCYT-7 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ACE-2			
B	Albumin				AMICA				Angiopoietin-4 (ANG-4)			
C	BAFF				CA19-9				CD163			
D	Clusterin				CRTAM				CXCL14 (BRAK)			
E	Cystatin C				Decorin				Dkk-3			
F	DLL1				Fetuin A				aFGF (FGF-1)			
G	FOLR1				Furin				GASP-1			
H	GASP-2				G-CSF R (CD114)				HAI-2			
I	IL-17B R (IL-17 RB)				IL-27				LAG-3			
J	LDL R				Pepsinogen I (PG1)				RANK			
K	RBP4				SOST				Syndecan-1			
L	TACI				TFPI				Thrombospondin 1			
M	TRAIL R2				TRANCE				Troponin I			
N	uPA				VE-Cadherin (CDH5)				WISP-1 (CCN4)			

QAH-CYT-8

(hCYT-8 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ANGPTL3			
B	beta IG-H3				CA9				Cathepsin B			
C	CD23				CHI3L1				CTLA4			
D	Dkk-4				DPPIV				EDA-A2			
E	Epo R				FGF-6				FGF-9			
F	Gas1				IGFBP-5				IL-1F5			
G	IL-1F6				IL-1F7				IL-1F8			
H	IL-1F9				IL-1F10				IL-1R5			
I	IL-17C				IL-18				IL-20			
J	IL-34				IL-5 R alpha				IL-10 R alpha			
K	Layilin				Leptin R				Marapsin			
L	Mer				MMP-7				P-Cadherin			
M	Prostasin				PSMA				SIGIRR			
N	TGF beta RIII				Tissue Factor (TF)				TWEAK			

QAH-CYT-9

(hCYT-9 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAMTS13			
B	Aggrecan				Angiotensinogen (AGT)				B7-H1 (CD274)			
C	BMPR-IA (ALK-3)				BMPR-II				Cadherin-11			
D	CD27 (TNFRSF7)				CD6				Ck beta 8-1 (CCL23)			
E	CNTF				DNAM-1 (CD226)				EMMPRIN (CD147)			
F	FLRG				Follistatin-like 1 (FSL1)				Fractalkine (CX3CL1)			
G	Galectin-1				GITR Ligand				Granulysin (LAG-2)			
H	IL-1 R3 (IL-1 R Acp)				IL-15 R alpha				IL-17E (IL-25)			
I	IL-32 alpha				L1CAM-2 (CHL-1)				LRIG3			
J	LRP-6				MEPE (OF45)				Nectin-4			
K	Periostin				Persephin				Renin			
L	RGM-B				ROBO3				S100A8			
M	Siglec-7 (CD328)				Syndecan-3				Thrombospondin 2			
N	Thrombospondin 5				Tie-1				ULBP-2			

QAH-CYT-10

(hCYT-10 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAM8			
B	ADAM12				B7-H3 (CD276)				BMPR-IB			
C	Cadherin-4				Cadherin-13				CD48 (SLAMF2)			
D	CD58 (LFA-3)				CD84 (SLAMF5)				CD99			
E	CD155 (PVR)				CD229 (SLAM3)				CEACAM-5			
F	CF XIV				Cystatin A				Cystatin B			
G	Cystatin E/M				Desmoglein 2				DR3 (TNFRSF25)			
H	ErbB4 (HER4)				ESAM				FGF-21			
I	Galectin-2				Galectin-9				ICOS			
J	JAM-A (CD321)				JAM-B (CD322)				Kallikrein 5			
K	Midkine				Pentraxin 3				Pref-1 (DLK-1)			
L	Siglec-10				SLAM (CD150)				SP-D			
M	Syndecan-4				Testican 2 (SPOCK2)				TIM-3 (KIM-3)			
N	TLR4				TRAIL (TNFSF10)				ULBP-1			

QAH-CYT-11

(hCYT-11 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ALK-1			
B	B7-H2				BLAME				BMP-8			
C	CD28				Common beta Chain				Contactin-1			
D	Desmoglein-1				Desmoglein-3				EDAR			
E	EphA1				EphB6				Ephrin-B3			
F	Epiregulin				FGF-12				FGF-17			
G	FOLR2				Galectin-8				GHR			
H	Glypican 1				Glypican 5				IFN-gamma R1			
I	IL-22 R alpha 1				IL-22BP				IL-23 R			
J	IL-31 RA				IL-7 R alpha				Integrin alpha 5			
K	MDM2				Nectin-1				NKp30			
L	Nogo Receptor				Notch-3				OSM R beta			
M	Prolactin R				RELT				Ryk			
N	Semaphorin 6D				Semaphorin 7A				Siglec-11			

QAH-CYT-12

(hCYT-12 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				B7-2			
B	BAFF R				Calcitonin				Calsyntenin-1			
C	Cathepsin E				cIAP-2				Coagulation Factor VII			
D	Complement MASP3				Endocan				EphA2			
E	EphB4				Ephrin-A4				FGF-23			
F	FGF-5				Flt-3				GLP-1			
G	Glypican 2				GM-CSF R alpha				GP73			
H	HTRA2				IL-20 R alpha				IL-4 R alpha			
I	JAM-C				Luteinizing hormone (LH)				Matrilin-3			
J	Meprin alpha				MSP R				N-Cadherin			
K	Neprilysin-2				NKp44				PAPP-A			
L	Pepsinogen II				Presenilin 1				PTH			
M	PYY				SOX2				TFF3			
N	TFPI-2				TRACP				Ubiquitin+1			

QAH-CYT-13

(hCYT-13 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ACE			
B	Activin RIB				ADAM23				Artemin			
C	Cardiotrophin-1				Cathepsin V				FABP1			
D	FGF-20				GDF-8				HAI-1			
E	IL-27 R alpha				Insulin R				Kallikrein 7			
F	LIF R alpha				Lipocalin-1				LTbR			
G	Mesothelin				MFRP				Neuropilin-2			
H	Neurturin				Nidogen-2				Olfactomedin-2			
I	p53				PD-ECGF				PDGF-CC			
J	Progranulin				Ret				ROBO4			
K	Semaphorin 6B				Serpin F1				SREC-I			
L	SREC-II				TLR1				TLR3			
M	TPP1				TREM-2				TrkC			
N	TROY				Uromodulin				XIAP			

QAH-CYT-14

(hCYT-14 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				4-1BB Ligand			
B	Activin RIIB				Aminopeptidase P2				BAMBI			
C	BOC				Brevican				Carbonic Anhydrase XII			
D	Carboxypeptidase A2				CD300c				CD320			
E	CDNF				CDO				CHST1			
F	CHST4				CILP-1				CNTF R alpha			
G	CRIM1				CRTAC1				CXADR			
H	Dopa Decarboxylase				DPPII				DSPG3			
I	EMR2				FCAR				FCRL1			
J	FCRL2				Gas6				GPR56			
K	GPVI				Hepsin				ILT2			
L	Jagged 2				Kirrel3				KLF4			
M	LAIR1				LAMP				LAMP1			
N	MDGA1				MIS RII				Neurexin 3 beta			

QAH-CYT-15

(hCYT-15 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AMIGO			
B	Aminopeptidase LRAP				Amnionless				Arylsulfatase A			
C	Bcl-w				CD109				CD157			
D	CD34				CD83				CLEC-1			
E	CLEC10A				CMG-2				CREG			
F	Cystatin SN				Cytokeratin-8				Dectin-1			
G	Desmocollin-3				Endoglycan				Galectin-4			
H	HAPLN1				Jagged 1				Langerin			
I	Lumican				Matriptase				MEP1B			
J	Nectin-3				OX40				OX40 Ligand			
K	p27				Pappalysin-2				Plexin B3			
L	Plexin D1				proGRP				PSA-total			
M	Reg1B				RGM-A				ROBO2			
N	Spinesin				TWEAK R				ULBP-3			

QAH-CYT-16

(hCYT-16 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin RIIA			
B	Biglycan				CA13				CA2			
C	CA72-4				CLEC-2				C-myc			
D	Cystatin D				Erythropoietin				FCRL5			
E	FGF-16				GATA-4				GFR alpha-1			
F	GFR alpha-2				Granzyme B				Granzyme H			
G	HIF-1a				htPAPP-A				IFNb			
H	IL-17 RC				IL-19				IL-20 R beta			
I	IL-22				ILT4				LAIR2			
J	LSECtin				Netrin-4				Norrin			
K	NRG1a				PD-L2				PDX-1			
L	Podocalyxin				RGM-C				S100A1			
M	Semaphorin 6A				SLITRK5				SR-AI			
N	ST6GAL1				Thyroid Peroxidase				Troponin C			

QAH-CYT-17

(hCYT-17 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Activin RIA			
B	ASAHL				B4GalT1				BAI1			
C	Brorin				C1qTNF4				CA14			
D	CA4				CA6				CA8			
E	Cadherin-6				Caspr2				CD27 Ligand			
F	CD300a				CD300e				CD300f			
G	CD4				CD5				CD69			
H	CK18				CK19				CPB1			
I	CRISP-2				DDR1				FUT8			
J	MIA				NTAL				NTB-A			
K	OMgp				PEAR1				Podoplanin			
L	PTH1R				Reg4				ROR1			
M	Semaphorin 4G				Serpin A5				Serpin B6			
N	Siglec-1				Sirtuin 2				Sirtuin 5			

QAH-CYT-18

(hCYT-18 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ANGPTL7			
B	CD36				CLEC9a				CL-P1			
C	Dectin-2				DLL4				DSCAM			
D	EDIL3				ENPP-7				Enteropeptidase			
E	FCRL3				FCRLB				FGF-3			
F	FLRT1				FLRT2				GBA3			
G	GDF-11				Glycoprotein V				Granzyme A			
H	IGSF4B				IL-28 R alpha				Kynureninase			
I	LAMA4				LRRC4				LRRTM3			
J	NG2				NQO-1				PCSK2			
K	PILR-alpha				Plexin A4				POGLUT1			
L	PRELP				Smad4				SOX15			
M	SOX7				SOX9				Syntaxin 6			
N	TROP-2				TSLP R				UNC5H4			

QAH-CYT-19

(hCYT-19 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAM22			
B	ARSB				B3GNT2				CA5B			
C	Caspase 7				Caspase 8				CD11b			
D	CD172g				CD39L2				CD39L4			
E	CD49b				CD7				CEACAM-3			
F	CPE				FABP6				FAM3C			
G	GDF-3				GSTM1				Kallikrein 11			
H	Kallikrein 12				Kremen-2				OSCAR			
I	PTP1B				Reg3A				R-Spondin 2			
J	S100A13				Semaphorin 4C				Sirtuin 1			
K	SMPD1				Sortilin				SPINK1			
L	Stabilin-2				SULT2A1				TCN2			
M	THSD1				TrkA				UCH-L3			
N	VAP-A				vWF-A2				Wnt-4			

QAH-CYT-20

(hCYT-20 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADAMTSL-1			
B	AMSH				Annexin V				BATF3			
C	Bora				Cadherin-17				Caveolin-2			
D	CD2				CD200 R1				CHST3			
E	COMT				Cystatin SA				DBH			
F	Desmin				EXTL3				Ficolin-1			
G	FosB				FRS2				GATA-5			
H	GFAP				GLI-3				HepaCAM			
I	HIF-1 beta				HSD17B1				IDO			
J	Kallikrein 1				Kell				MDL-1			
K	NPDC-1				Numb				Olig2			
L	p63				Pax3				Semaphorin 4D			
M	SPHK1				TAZ				TC-PTP			
N	TGM3				TPST2				TREML1			

QAH-CYT-21

(hCYT-21 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				Cf10			
B	CHMP2B				Contactin-3				Cortactin			
C	CrkL				Cyr61				DAPP1			
D	DCTN1				DFF45				DRAK1			
E	GRAP2				GRK5				HAO-1			
F	LRIG1				MMP-12				NCK1			
G	Nectin-2				Nesfatin-1				Neurogranin			
H	Nrf2				NUDT5				NUP85			
I	PAR1				PP				PRX2			
J	PSMA1				PU.1				RalA			
K	RCOR1				Serpin B8				SH2D1A			
L	SHP-1				Siglec-6				SorCS3			
M	THAP11				ULBP-4				UNC5H3			
N	VAMP-1				VAMP-2				Visfatin			

QAH-CYT-22

(hCYT-22 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				C1qTNF9			
B	CA5A				CANT1				Cathepsin H			
C	Contactin-5				CTRC				Draxin			
D	EphB2				EphB3				FABP8			
E	Fgr				FKBP51				FUCA1			
F	Galanin				GALNT10				GKN1			
G	Glyoxalase II				HS3ST1				HS3ST3B1			
H	Lin28				LOXL2				LRRTM4			
I	MAP1D				Matrilin-2				MCEMP1			
J	Mcl-1				MDGA2				MEF2C			
K	METAP2				Neurocan				Nogo-A			
L	PCK1				PlGF-2				PON1			
M	SALM4				Semaphorin 6C				SorCS2			
N	ST3GAL1				ST8SIA1				TSK			

QAH-CYT-23

(hCYT-23 Map) Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				ADA			
B	AIF				AKR1C4				ASAH2			
C	BCL-2				BID				Calreticulin			
D	Calreticulin-2				CD314				CD39L3			
E	CD51				CD99-L2				CDC25B			
F	Cerberus 1				CHST2				Cochlin			
G	CRELD2				DC-SIGNR				eNOS			
H	ENPP-2				FABP4				FcERI			
I	FGF R5				GALNT2				GALNT3			
J	GIF				GPR111				GUSB			
K	Inhibin A				LILRB4				Neuroglycan C			
L	NKp46				NPTXR				ROR2			
M	SCCA2				Siglec-2				SIRP alpha			
N	SorCS1				Trypsin 1				Trypsin 3			

QAH-CYT-24

(hCYT-24 Map) Each antibody is printed in quadruplicate horizontally												
	1	2	3	4	1	2	3	4	1	2	3	4
A	POS1				POS2				AMIGO2			
B	Arginase 1				B7-H4				Bcl-10			
C	CD42b				CD73				CES1			
D	CES2				cIAP-1				Cyclophilin A			
E	Cystatin S				DNMT3A				Epimorphin			
F	GDF-9				Glypican 3				GPR115			
G	HE4				HO-1				HS3ST4			
H	IGSF3				IL-17 RD				Integrin alpha 1			
I	KIR2DL3				LAMP2				LEDGF			
J	MOG				Nestin				Neudesin			
K	Neuroligin 2				NKp80				Osteoadherin			
L	PDGF R alpha				PRDX4				Syntaxin 4			
M	TAFA1				TAFA2				TAFA5			
N	Tenascin R				TGM4				TMEFF1			

X. Standard Concentrations

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

QAH-INF-3	(pg/ml)	QAH-GF-1	(pg/ml)	QAH-CHE-1	(pg/ml)	QAH-REC-1	(pg/ml)	QAH-CYT-4	(pg/ml)
BLC	2,000	AR	10,000	6Ckine	40,000	4-1BB	10,000	Activin A	100,000
Eotaxin	4,000	BDNF	2,000	Axl	4,000	ALCAM	10,000	AgRP	10,000
Eotaxin-2	1,000	bFGF	20,000	BTC	20,000	B7-1	10,000	ANG	2,000
G-CSF	20,000	BMP-4	100,000	CCL28	40,000	BCMA	20,000	ANG-1	40,000
GM-CSF	1,000	BMP-5	100,000	CTACK	50,000	CD14	10,000	Angiostatin	1,000,000
I-309	4,000	BMP-7	40,000	CXCL16	20,000	CD30	10,000	Catheprin S	10,000
ICAM-1	100,000	b-NGF	10,000	ENA-78	10,000	CD40 L	10,000	CD 40	10,000
IFNg	2,000	EGF	200	Eotaxin-3	20,000	CEACAM-1	10,000	Cripto-1	10,000
IL-1a	2,000	EGF R	10,000	GCP-2	10,000	DR6	4,000	DAN	40,000
IL-1b	1,000	EG-VEGF	10,000	GRO	1,000	Dtk	20,000	DKK-1	80,000
IL-1ra	2,000	FGF-4	100,000	HCC-1	4,000	Endoglin	4,000	E-Cadherin	80,000
IL-2	2,000	FGF-7	10,000	HCC-4	10,000	ErbB3	20,000	EpCAM	20,000
IL-4	2,000	GDF-15	2,000	IL-9	200,000	E-Selectin	40,000	FAS L	2,000
IL-5	4,000	GDNF	4,000	IL-17F	100,000	Fas	2,000	Fcr RIIBC	10,000
IL-6	2,000	GH	10,000	IL-18 BPa	60,000	Flt-3L	2,000	Follistatin	40,000
IL-6sR	10,000	HB-EGF	10,000	IL-28A	10,000	GITR	10,000	Galectin-7	100,000
IL-7	4,000	HGF	4,000	IL-29	100,000	HVEM	40,000	ICAM-2	100,000
IL-8	500	IGFBP-1	5,000	IL-31	40,000	ICAM-3	100,000	IL-13 R1	10,000
IL-10	4,000	IGFBP-2	20,000	IP-10	10,000	Contactin-2	100,000	IL-13 R2	20,000
IL-11	20,000	IGFBP-3	200,000	I-TAC	10,000	IL-1 RI	4,000	IL-17B	40,000
IL-12p40	10,000	IGFBP-4	200,000	LIF	13,000	IL-2 Rg	10,000	IL-2 Ra	10,000
IL-12p70	500	IGFBP-6	100,000	LIGHT	10,000	IL-10 Rb	4,000	IL-2 Rb	100,000
IL-13	1,000	IGF-I	20,000	Lymphotactin	100,000	IL-17R	10,000	IL-23	40,000
IL-15	4,000	Insulin	20,000	MCP-2	2,000	IL-21R	20,000	LAP	4,000
IL-16	5,000	MCSF R	40,000	MCP-3	4,000	LIMPII	4,000	NrCAM	20,000
IL-17	4,000	NGF R	10,000	MCP-4	10,000	Lipocalin-2	1,000	PAI-I	40,000
MCP-1	2,000	NT-3	40,000	MDC	10,000	L-Selectin	100,000	PDGF-AB	10,000
MCSF	4,000	NT-4	10,000	MIF	4,000	LYVE-1	2,000	Resistin	20,000
MIG	5,000	OPG	4,000	MIP-3a	4,000	MICA	10,000	SDF-1b	40,000
MIP-1a	10,000	PDGF-AA	10,000	MIP-3b	20,000	MICB	15,000	sgp130	80,000
MIP-1b	1,000	PIGF	4,000	MPIF-1	10,000	NRG1-b1	15,000	Shh N	40,000
MIP-1d	10,000	SCF	10,000	MSPa	100,000	PDGF Rb	100,000	Siglec-5	10,000
PDGF-BB	2,000	SCF R	20,000	NAP-2	4,000	PECAM-1	20,000	ST2	4,000
RANTES	20,000	TGFa	10,000	OPN	100,000	RAGE	10,000	TGF-b2	40,000
TIMP-1	40,000	TGFb1	100,000	PARC	4,000	TIM-1	10,000	Tie-2	10,000
TIMP-2	40,000	TGFb3	40,000	PF4	100,000	TRAIL R3	5,000	TPO	200,000
TNFa	2,000	VEGF	10,000	SDF-1a	10,000	Trappin-2	10,000	TRAIL R4	8,000
TNFb	20,000	VEGF R2	10,000	TARC	10,000	uPAR	40,000	TREM-1	20,000
TNF RI	40,000	VEGF R3	40,000	TECK	100,000	VCAM-1	200,000	VEGF-C	20,000
TNF RII	40,000	VEGF-D	20,000	TSLP	10,000	XEDAR	10,000	VEGF R1	40,000

QAH-CYT-5	(pg/ml)	QAH-CYT-6	(pg/ml)	QAH-CYT-7	(pg/ml)	QAH-CYT-8	(pg/ml)	QAH-CYT-9	(pg/ml)
Adiponectin	100,000	2B4	10,000	ACE-2	400,000	ANGPTL3	10,000	ADAMTS13	100,000
Adipsin	20,000	ADAM-9	100,000	Albumin	20,000	bIG-H3	10,000	Aggrecan	20,000
AFP	10,000	ANG-2	20,000	AMICA	20,000	CA9	10,000	Angiotensinogen	100,000
ANGPTL4	400,000	APRIL	200,000	ANG-4	20,000	Cathepsin B	10,000	B7-H1	10,000
B2M	10,000	BMP-2	100,000	BAFF	10,000	CD23	10,000	BMPR-IA	100,000
BCAM	40,000	BMP-9	4,000	CA19-9	100,000	CHI3L1	10,000	BMPR-II	100,000
CA125	100,000	C5a	10,000	CD163	200,000	CTLA4	4,000	Cadherin-11	400,000
CA15-3	30,000	Cathepsin L	10,000	Clusterin	10,000	Dkk-4	100,000	CD27	10,000
CEA	20,000	CD200	100,000	CRTAM	4,000	DPPIV	200,000	CD6	100,000
CRP	10,000	CD97	100,000	CXCL14	100,000	EDA-A2	10,000	Ck beta 8-1	100,000
ErbB2	10,000	Chemerin	200,000	Cystatin C	100,000	Epo R	40,000	CNTF	100,000
Ferritin	800,000	DcR3	200,000	Decorin	2,000	FGF-6	10,000	DNAM-1	100,000
FSH	10,000	FABP2	100,000	Dkk-3	100,000	FGF-9	4,000	EMMPRIN	2,000
GROa	100,000	FAP	20,000	DLL1	20,000	Gas1	100,000	FLRG	10,000
hCGb	20,000	FGF-19	20,000	Fetuin A	100,000	IGFBP-5	200,000	Follistatin-like 1	400,000
IGF-I SR	100,000	Galectin-3	4,000	aFGF	200,000	IL-1F5	200,000	Fractalkine	40,000
IL-1 sRII	20,000	HGF R	4,000	FOLR1	100,000	IL-1F6	200,000	Galectin-1	20,000
IL-3	10,000	IFNab R2	100,000	Furin	200,000	IL-1F7	100,000	GITR Ligand	200,000
IL-18 Rb	20,000	IGF-II	100,000	GASP-1	2,000	IL-1F8	4,000	Granulysin	4,000
IL-21	100,000	IGF-II R	20,000	GASP-2	100,000	IL-1F9	100,000	IL-1 R3	10,000
Leptin	40,000	IL-1 R6	100,000	G-CSF R	10,000	IL-1F10	200,000	IL-15 R	2,000
MMP-1	40,000	IL-24	100,000	HAI-2	40,000	IL-1R5	1,000	IL-17E	40,000
MMP-2	100,000	IL-33	10,000	IL-17B R	100,000	IL-17C	400,000	IL-32 alpha	4,000
MMP-3	40,000	Kallikrein 14	4,000	IL-27	10,000	IL-18	40,000	L1CAM-2	200,000
MMP-8	10,000	Legumain	10,000	LAG-3	100,000	IL-20	100,000	LRIG3	200,000
MMP-9	20,000	LOX-1	2,000	LDL R	2,000	IL-34	40,000	LRP-6	200,000
MMP-10	10,000	MBL	1,000	Pepsinogen I	20,000	IL-5 Ra	400,000	MEPE	200,000
MMP-13	10,000	Neprilysin	20,000	RANK	100,000	IL-10 Ra	200,000	Nectin-4	20,000
NCAM-1	200,000	Notch-1	4,000	RBP4	20,000	Layilin	10,000	Periostin	200,000
Nidogen-1	20,000	NOV	4,000	SOST	40,000	Leptin R	100,000	Persephin	100,000
NSE	100,000	Osteoactivin	10,000	Syndecan-1	100,000	Marapsin	20,000	Renin	10,000
OSM	10,000	PD-1	4,000	TACI	40,000	Mer	10,000	RGM-B	100,000
Procalcitonin	100,000	PGRP-5	1,000	TFPI	100,000	MMP-7	100,000	ROBO3	2,000
Prolactin	400,000	Serpin A4	10,000	TSP-1	100,000	P-Cadherin	100,000	S100A8	10,000
PSA	20,000	sFRP-3	100,000	TRAIL R2	4,000	Prostasin	20,000	Siglec-7	2,000
Siglec-9	40,000	Thrombomodulin	100,000	TRANCE	40,000	PSMA	100,000	Syndecan-3	100,000
TACE	100,000	TLR2	20,000	Troponin I	200,000	SIGIRR	100,000	Thrombospondin	10,000
Thyroglobulin	100,000	TRAIL R1	10,000	uPA	4,000	TGFb RIII	20,000	Thrombospondin	10,000
TIMP-4	20,000	Transferrin	100,000	VE-Cadherin	200,000	TF	4,000	Tie-1	10,000
TSH	20,000	WIF-1	20,000	WISP-1	200,000	TWEAK	100,000	ULBP-2	4,000

QAH-CYT-10	(pg/ml)	QAH-CYT-11	(pg/ml)	QAH-CYT-12	(pg/ml)	QAH-CYT-13	(pg/ml)	QAH-CYT-14	(pg/ml)
ADAM8	100,000	ALK-1	10,000	B7-2	2,000	ACE	100,000	4-1BB Ligand	40,000
ADAM12	20,000	B7-H2	2,000	BAFF R	10,000	Activin RIB	20,000	Activin RIIB	20,000
B7-H3	4,000	BLAME	400,000	Calcitonin	100,000	ADAM23	10,000	Aminopeptidase	100,000
BMPR-IB	10,000	BMP-8	40,000	Calsyntenin-1	40,000	Artemin	10,000	BAMBI	4,000
Cadherin-4	10,000	CD28	20,000	Cathepsin E	100,000	Cardiotrophin-1	40,000	BOC	20,000
Cadherin-13	100,000	Common beta Ch	10,000	cIAP-2	200,000	Cathepsin V	10,000	Brevican	1,000
CD48	200,000	Contactin-1	4,000	Coagulation Factc	20,000	FABP1	400,000	Carbonic Anhydra	100,000
CD58	100,000	Desmoglein-1	10,000	Complement MA:	100,000	FGF-20	10,000	Carboxypeptidase	4,000
CD84	100,000	Desmoglein-3	10,000	Endocan	1,000	GDF-8	100,000	CD300c	4,000
CD99	4,000	EDAR	4,000	EphA2	20,000	HAI-1	100,000	CD320	10,000
CD155	100,000	EphA1	10,000	EphB4	20,000	IL-27 Ra	10,000	CDNF	1,000
CD229	10,000	EphB6	4,000	Ephrin-A4	20,000	Insulin R	40,000	CDO	4,000
CEACAM-5	100,000	Ephrin-B3	100,000	FGF-23	10,000	Kallikrein 7	4,000	CHST1	20,000
CF XIV	20,000	Epiregulin	800,000	FGF-5	100,000	LIF R alpha	10,000	CHST4	40,000
Cystatin A	4,000	FGF-12	2,000	Flt-3	10,000	Lipocalin-1	1,000	CILP-1	4,000
Cystatin B	4,000	FGF-17	20,000	GLP-1	20,000	LTbR	400	CNTF R alpha	1,000
Cystatin E/M	10,000	FOLR2	20,000	Glypican 2	100,000	Mesothelin	4,000	CRIM1	1,000
Desmoglein 2	20,000	Galectin-8	1,000	GM-CSF R alpha	100,000	MFRP	40,000	CRTAC1	20,000
DR3	100,000	GHR	4,000	GP73	10,000	Neuropilin-2	4,000	CXADR	2,000
ErbB4	10,000	Glypican 1	10,000	HTRA2	100,000	Neurturin	10,000	Dopa Decarboxyla	1,000
ESAM	10,000	Glypican 5	10,000	IL-20 R alpha	100,000	Nidogen-2	20,000	DPPII	10,000
FGF-21	4,000	IFN-gamma R1	1,000	IL-4 R alpha	10,000	Olfactomedin-2	40,000	DSPG3	20,000
Galectin-2	20,000	IL-22 R alpha 1	4,000	JAM-C	10,000	p53	10,000	EMR2	4,000
Galectin-9	10,000	IL-22BP	100,000	Luteinizing hormc	10,000	PD-ECGF	10,000	FCAR	40,000
ICOS	100,000	IL-23 R	4,000	Matrilin-3	2,000	PDGF-CC	40,000	FCRL1	40,000
JAM-A	4,000	IL-31 RA	10,000	Meprin alpha	100,000	Progranulin	10,000	FCRL2	40,000
JAM-B	10,000	IL-7 R alpha	2,000	MSP R	10,000	Ret	40,000	Gas6	4,000
Kallikrein 5	10,000	Integrin alpha 5	200,000	N-Cadherin	100,000	ROBO4	4,000	GPR56	10,000
Midkine	20,000	MDM2	20,000	Neprilysin-2	100,000	Semaphorin 6B	10,000	GPVI	1,000
Pentraxin 3	10,000	Nectin-1	100,000	NKp44	2,000	Serpin F1	10,000	Hepsin	10,000
Pref-1	100,000	NKp30	10,000	PAPP-A	100,000	SREC-I	4,000	ILT2	1,000
Siglec-10	200,000	Nogo Receptor	40,000	Pepsinogen II	10,000	SREC-II	20,000	Jagged 2	40,000
SLAM	100,000	Notch-3	10,000	Presenilin 1	10,000	TLR1	4,000	Kirrel3	20,000
SP-D	20,000	OSM R beta	100,000	PTH	20,000	TLR3	1,000	KLF4	20,000
Syndecan-4	1,000	Prolactin R	10,000	PYY	40,000	TPP1	10,000	LAIR1	100,000
Testican 2	40,000	RELT	10,000	SOX2	100,000	TREM-2	4,000	LAMP	40,000
TIM-3	10,000	Ryk	10,000	TFF3	100,000	TrkC	4,000	LAMP1	40,000
TLR4	200,000	Semaphorin 6D	100,000	TFPI-2	100,000	TROY	40,000	MDGA1	10,000
TRAIL	2,000	Semaphorin 7A	20,000	TRACP	100,000	Uromodulin	4,000	MIS RII	10,000
ULBP-1	20,000	Siglec-11	200,000	Ubiquitin+1	100,000	XIAP	10,000	Neurexin 3 beta	4,000

QAH-CYT-15	(pg/ml)	QAH-CYT-16	(pg/ml)	QAH-CYT-17	(pg/ml)	QAH-CYT-18	(pg/ml)	QAH-CYT-19	(pg/ml)
AMIGO	100,000	Activin RIIA	40,000	Activin RIA	20,000	ANGPTL7	4,000	ADAM22	20,000
Aminopeptidase	200,000	Biglycan	10,000	ASAHL	40,000	CD36	20,000	ARSB	10,000
Amnionless	40,000	CA13	10,000	B4GalT1	40,000	CLEC9a	4,000	B3GNT2	20,000
Arylsulfatase A	100,000	CA2	100,000	BAI1	20,000	CL-P1	40,000	CA5B	100,000
Bcl-w	1,000	CA72-4	10,000	Brorin	20,000	Dectin-2	4,000	Caspase 7	200,000
CD109	100,000	CLEC-2	100,000	C1qTNF4	40,000	DLL4	100,000	Caspase 8	40,000
CD157	1,000	C-myc	20,000	CA14	2,000	DSCAM	10,000	CD11b	40,000
CD34	40,000	Cystatin D	4,000	CA4	20,000	EDIL3	40,000	CD172g	20,000
CD83	10,000	Erythropoietin	40,000	CA6	20,000	ENPP-7	2,000	CD39L2	20,000
CLEC-1	200,000	FCRL5	100,000	CA8	40,000	Enteropeptidase	2,000	CD39L4	40,000
CLEC10A	10,000	FGF-16	10,000	Cadherin-6	4,000	FCRL3	4,000	CD49b	100,000
CMG-2	40,000	GATA-4	20,000	Caspr2	10,000	FCRLB	10,000	CD7	20,000
CREG	100,000	GFR alpha-1	10,000	CD27 Ligand	10,000	FGF-3	100,000	CEACAM-3	20,000
Cystatin SN	100,000	GFR alpha-2	4,000	CD300a	10,000	FLRT1	10,000	CPE	10,000
Cytokeratin-8	100,000	Granzyme B	10,000	CD300e	200,000	FLRT2	10,000	FABP6	40,000
Dectin-1	10,000	Granzyme H	10,000	CD300f	2,000	GBA3	20,000	FAM3C	100,000
Desmocollin-3	20,000	HIF-1a	40,000	CD4	200,000	GDF-11	10,000	GDF-3	100,000
Endoglycan	200,000	htPAPP-A	100,000	CD5	20,000	Glycoprotein V	10,000	GSTM1	200,000
Galectin-4	1,000	IFNb	100,000	CD69	400	Granzyme A	1,000	Kallikrein 11	400
HAPLN1	200,000	IL-17 RC	10,000	CK18	40,000	IGSF4B	10,000	Kallikrein 12	4,000
Jagged 1	100,000	IL-19	100,000	CK19	40,000	IL-28 R alpha	1,000	Kremen-2	4,000
Langerin	40,000	IL-20 R beta	20,000	CPB1	40,000	Kynureninase	4,000	OSCAR	200,000
Lumican	10,000	IL-22	100,000	CRISP-2	10,000	LAMA4	10,000	PTP1B	20,000
Matriptase	100,000	ILT4	20,000	DDR1	40,000	LRRC4	10,000	Reg3A	4,000
MEP1B	40,000	LAIR2	100,000	FUT8	10,000	LRRTM3	100,000	R-Spondin 2	4,000
Nectin-3	40,000	LSECtin	20,000	MIA	100,000	NG2	20,000	S100A13	100
OX40	20,000	Netrin-4	200,000	NTAL	10,000	NQO-1	20,000	Semaphorin 4C	20,000
OX40 Ligand	40,000	Norrin	100,000	NTB-A	100,000	PCSK2	40,000	Sirtuin 1	100,000
p27	20,000	NRG1a	4,000	OMgp	10,000	PILR-alpha	400	SMPD1	100,000
Pappalysin-2	20,000	PD-L2	100,000	PEAR1	4,000	Plexin A4	10,000	Sortilin	100,000
Plexin B3	20,000	PDX-1	10,000	Podoplanin	10,000	POGLUT1	100,000	SPINK1	100,000
Plexin D1	200,000	Podocalyxin	10,000	PTH1R	10,000	PRELP	4,000	Stabilin-2	20,000
proGRP	100,000	RGM-C	100,000	Reg4	40,000	Smad4	4,000	SULT2A1	100,000
PSA-total	20,000	S100A1	100,000	ROR1	100,000	SOX15	1,000	TCN2	4,000
Reg1B	10,000	Semaphorin 6A	20,000	Semaphorin 4G	2,000	SOX7	100,000	THSD1	200,000
RGM-A	20,000	SLITRK5	100,000	Serpin A5	40,000	SOX9	40,000	TrkA	200,000
ROBO2	10,000	SR-AI	20,000	Serpin B6	10,000	Syntaxin 6	1,000	UCH-L3	200,000
Spinesin	100,000	ST6GAL1	100,000	Siglec-1	4,000	TROP-2	400	VAP-A	20,000
TWEAK R	20,000	Thyroid Peroxida:	100,000	Sirtuin 2	1,000	TSLP R	20,000	vWF-A2	4,000
ULBP-3	10,000	Troponin C	100,000	Sirtuin 5	40,000	UNC5H4	1,000	Wnt-4	40,000

QAH-CYT-20	(pg/ml)	QAH-CYT-21	(pg/ml)	QAH-CYT-22	(pg/ml)	QAH-CYT-23	(pg/ml)	QAH-CYT-24	(pg/ml)
ADAMTSL-1	100,000	Cf10	100,000	C1qTNF9	10,000	ADA	100,000	AMIGO2	20,000
AMSH	10,000	CHMP2B	100,000	CA5A	10,000	AIF	40,000	Arginase 1	10,000
Annexin V	100,000	Contactin-3	100,000	CANT1	100,000	AKR1C4	100,000	B7-H4	100,000
BATF3	100,000	Cortactin	40,000	Cathepsin H	20,000	ASAH2	100,000	Bcl-10	100,000
Bora	100,000	CrkL	20,000	Contactin-5	10,000	BCL-2	100,000	CD42b	100,000
Cadherin-17	40,000	Cyr61	4,000	CTRC	4,000	BID	100,000	CD73	10,000
Caveolin-2	10,000	DAPP1	40,000	Draxin	1,000	Calreticulin	40,000	CES1	2,000
CD2	40,000	DCTN1	100,000	EphB2	100,000	Calreticulin-2	100,000	CES2	50,000
CD200 R1	20,000	DFF45	10,000	EphB3	10,000	CD314	100,000	cIAP-1	100,000
CHST3	100,000	DRAK1	100,000	FABP8	100,000	CD39L3	40,000	Cyclophilin A	10,000
COMT	100,000	GRAP2	40,000	Fgr	100,000	CD51	40,000	Cystatin S	400
Cystatin SA	20,000	GRK5	100,000	FKBP51	100,000	CD99-L2	20,000	DNMT3A	20,000
DBH	100,000	HAO-1	100,000	FUCA1	40,000	CDC25B	100,000	Epimorphin	4,000
Desmin	20,000	LRIG1	40,000	Galanin	100,000	Cerberus 1	10,000	GDF-9	40,000
EXTL3	10,000	MMP-12	20,000	GALNT10	40,000	CHST2	40,000	Glypican 3	100,000
Ficolin-1	10,000	NCK1	100,000	GKN1	100,000	Cochlin	100,000	GPR115	100,000
FosB	10,000	Nectin-2	4,000	Glyoxalase II	100,000	CRELD2	40,000	HE4	1,000
FRS2	20,000	Nesfatin-1	100,000	HS3ST1	10,000	DC-SIGNR	100,000	HO-1	100
GATA-5	20,000	Neurogranin	20,000	HS3ST3B1	10,000	eNOS	100,000	HS3ST4	100,000
GFAP	200,000	Nrf2	10,000	Lin28	4,000	ENPP-2	100,000	IGSF3	20,000
GLI-3	4,000	NUDT5	100,000	LOXL2	100,000	FABP4	100,000	IL-17 RD	40,000
HepaCAM	2,000	NUP85	100,000	LRRTM4	2,000	FcERI	2,000	Integrin alpha 1	30,000
HIF-1 beta	100,000	PAR1	100,000	MAP1D	10,000	FGF R5	2,000	KIR2DL3	40,000
HSD17B1	10,000	PP	100,000	Matrilin-2	4,000	GALNT2	100,000	LAMP2	100,000
IDO	100,000	PRX2	20,000	MCEMP1	10,000	GALNT3	100,000	LEDGF	40,000
Kallikrein 1	40,000	PSMA1	20,000	Mcl-1	10,000	GIF	100,000	MOG	4,000
Kell	100,000	PU.1	10,000	MDGA2	200,000	GPR111	20,000	Nestin	20,000
MDL-1	20,000	RalA	40,000	MEF2C	2,000	GUSB	20,000	Neudesin	10,000
NPDC-1	200	RCOR1	10,000	METAP2	20,000	Inhibin A	40,000	Neuroligin 2	20,000
Numb	4,000	Serpin B8	40,000	Neurocan	2,000	LILRB4	40,000	NKp80	4,000
Olig2	100,000	SH2D1A	4,000	Nogo-A	1,000	Neuroglycan C	100,000	Osteoadherin	2,000
p63	40,000	SHP-1	100,000	PCK1	20,000	NKp46	2,000	PDGF R alpha	100,000
Pax3	100,000	Siglec-6	10,000	PlGF-2	400	NPTXR	40,000	PRDX4	100,000
Semaphorin 4D	100,000	SorCS3	100,000	PON1	100,000	ROR2	2,000	Syntaxin 4	40,000
SPHK1	100,000	THAP11	100,000	SALM4	10,000	SCCA2	40,000	TAFA1	100,000
TAZ	20,000	ULBP-4	100,000	Semaphorin 6C	40,000	Siglec-2	10,000	TAFA2	100,000
TC-PTP	100,000	UNC5H3	10,000	SorCS2	4,000	SIRP alpha	1,000	TAFA5	20,000
TGM3	4,000	VAMP-1	40,000	ST3GAL1	100,000	SorCS1	100,000	Tenascin R	100,000
TPST2	40,000	VAMP-2	100,000	ST8SIA1	10,000	Trypsin 1	100,000	TGM4	100,000
TREML1	4,000	Visfatin	40,000	TSK	100,000	Trypsin 3	2,000	TMEFF1	20,000

XI. Spiking & Recovery

Please view the individual array manuals for spiking & recovery data

XII. Quantibody® Q-Analyzer

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-CAA-X00-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

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

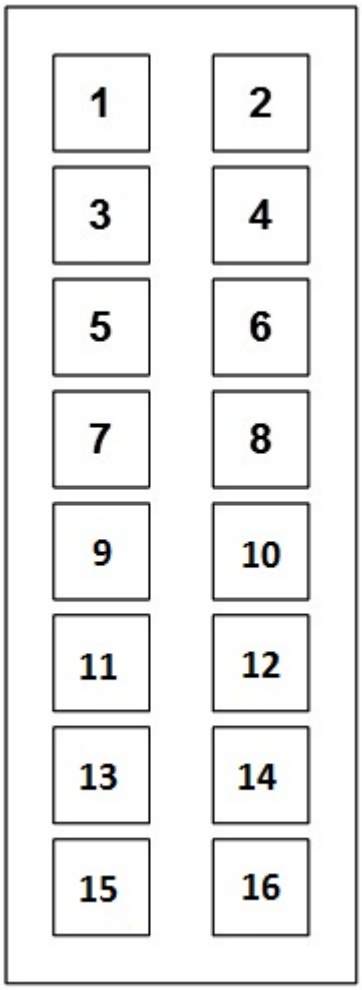
Date:_____

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, 200 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.html