Procarta® Cytokine Assay Kit

User Manual Specifically for measuring Cytokines in Tissue or Cell Lysates

Panomics I Affymetrix, Inc.

Procarta Cytokine Assay Kit User Manual

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Contents

About the User Manual

Who Should Read this Manual

Anyone that has purchased a Procarta Cytokine Assay Kit from Panomics to perform quantitative, multiplexed measurement of **Human, Mouse or Rat cytokines** derived from **Tissue or Cell Lysates** using the Luminex® Technology.

IMPORTANT Please note that we provide application specific manuals for cytokine measurement from **Cell Culture Supernatants** and **Serum/Plasma Samples**. Our most updated manuals can be obtained from our website.

What this Manual Covers

This manual provides recommendations and step-by-step procedures for the following:

- Sample and assay preparation
- Set up and operation of the vacuum manifold system
- Assay procedure
- Troubleshooting

Safety Warnings and Precautions

! WARNING ! Though the general procedure is very similar to other Luminex cytokine assays, there are subtle differences due to the approach we have taken in developing the assay to have an improved workflow, so please ensure that you do not refer to other vendors manuals and please read the supplied Panomics manual prior to starting the assay.

CAUTION All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

CAUTION This kit contains small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. When disposing, flush drains with a large volume of water to prevent azide accumulation. Observe all state and local regulations for disposal.

For More Information

For information about the Procarta products mentioned in this manual, visit our website at www.panomics.com.

About the Procarta Cytokine Assay Kit

Procarta Cytokine Assay Defined

The Procarta Cytokine assays are multiplex immunoassays based on xMAP® detection technology developed by Luminex. This bead based multiplex assay kit can quantitatively measure multiple cytokines from as little as 25 µL of Tissue or Cell Lysate in 3 hours with a sensitivity of less than 1 pg/ml/cytokine. For increased sensitivity, the sample input can be increased to 100 µL of Tissue or Cell Lysate.

Available Kit Formats

The cytokine panels for Procarta Cytokine Assay Kit are available as:

- Standard pre-mixed panels
- "By Request", user selected panels, provided in premixed and ready to use format

Procarta Cytokine Assay Kits are available as single or ten plate 96-well formats and contain all the reagents required to detect cytokines from cell culture supernatants. For detection of cytokines in plasma or serum, standard diluent kits are sold separately.

Procarta Cytokine Assay Kit Contents and Handling Conditions

Kit Contents and Storage

The Procarta Cytokine Assay Kit contains the following components. The kits are available as single plate or ten plate formats. Refer to the product insert for quantities and details of components supplied.

Procarta Cytokine Kit components:

Component	Storage
Reading Buffer	4 °C
10X Wash Buffer	4 °C
Detection Antibody, premixed	4 °C
Antigen Standards, premixed, lyophilized	4 °C
Streptavidin-PE (SAPE)	4 °C
Antibody Beads	4 °C
Filter Plate	4 °C
Plate Seals	4 °C
PCR 8 tube strip	4 °C

Kit Handling

- Store the entire kit at 4 °C
- Do not reuse or store resuspended antigen standards
- 10X Wash Buffer and its 1X dilution can be stored at 4 °C or room temperature for up to 6 months

Required Materials and Equipment Not Provided

Equipment

Item	Source
Vacuum filtration system	Millipore (P/N MAVM0960R and WP6111560)
Microplate shaker	Labline model 4625 or equivalent with 3 mm orbit
Luminex or Luminex-based instrument	MiraiBio, Bio-Rad or other Luminex instrument provider

Sample Type Specific Materials

When preparing Tissue Lysates for cytokine measurements, you will need to order the Procarta Lysis Buffer.

Catalog Number	Contents	Size
PC6002	Procarta Lysis Buffer	20 mL

Overview of Assay Workflow

Step 1. Prepare Samples and Reagents

a. Collect and prepare samples
b. Prepare antigen standards
c. Prepare 1X Wash Buffer

Step 2. Pre-wet Filter Plate

a. Let sit for 5 min
b. Filter

Step 3. Add Antibody Beads

a. Filter
b. Wash once
c. Filter

Step 4. Add samples/standards

a. Incubate 30 min shaking, 20-25° C
b. Wash/filter three times

Step 5. Add Detection Antibody

a. Incubate 30 min shaking, 20-25° C
b. Wash/filter three times

Step 6. Add Streptavidin-PE

a. Incubate 30 min shaking, 20-25° C
b. Wash/filter three times

Step 7. Read plate

Set-Up and Operation of the Vacuum Manifold System

About Using the Vacuum Manifold

This topic describes how to set up and use the Millipore vacuum manifold. This includes how to calibrate the pressure and important guidelines that will help to ensure good assay reproducibility.

We recommend that you set up and calibrate the manifold before you start the assay to ensure the assay is performed without interruption.

Sealing Filter Plates

- Lay a Plate Seal over the Filter Plate and roll a 5 mL serological pipet (or equivalent) over the Plate Seal to seal the Filter Plate.

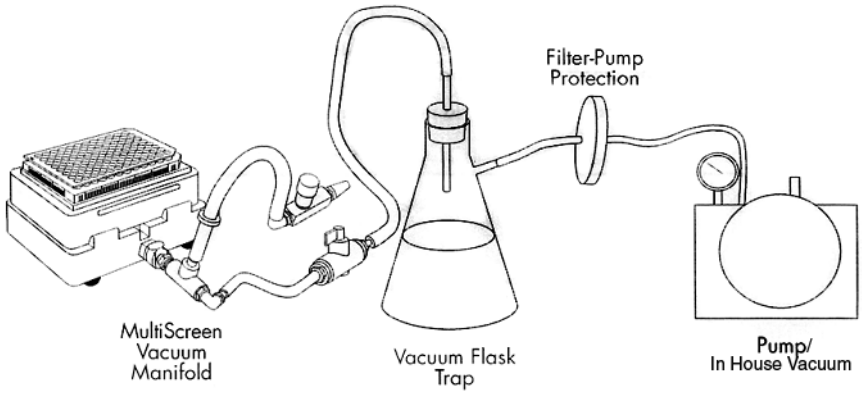
  This ensures adequate plate sealing while avoiding any leakage due to capillary action.

IMPORTANT To avoid Filter Plate leakages, do not seal Filter Plates using a rubber roller (or equivalent) as they apply significant pressure resulting in leakage.

- Seal all unused wells with an enclosed Plate Seal to ensure proper vacuum pressure.

Setting Up and Calibrating the Manifold

To set up and calibrate the manifold:

Step	Action
1	Set up the Filter Plate vacuum manifold as shown below. Follow the manufacturer's manual for details. 
2	Calibrate the vacuum pressure using the Filter Plate: a. Place the Filter Plate on top of the manifold. b. Turn on the vacuum. c. Press the corners of the Filter Plate to form a tight seal. d. Set the pressure to 2–3 mm of Hg. **IMPORTANT** If the vacuum is too high, beads will be trapped on the filter.

Operating the Manifold

To operate the manifold:

Step	Action
1	Once the vacuum is set correctly, remove the Filter Plate. Check vacuum calibration periodically. As a general guideline, 200 µL of solution should take approximately 8-10 seconds to clear the well of a Filter Plate.
2	For all filtration steps, turn the Filter Plate vacuum manifold on, transfer the Filter Plate to the vacuum manifold and then filter the solution. Avoid splashing and cross-contamination of wells during all wash steps. **IMPORTANT** During filtration, maintain the vacuum between 2–3 mm of Hg. Higher vacuum settings may result in Capture Bead loss. **IMPORTANT** Do not allow the Filter Plates to air-dry following washes. Immediately add the next component following each filtration step. **IMPORTANT** We recommend peforming all of the washes steps next to the manifold to minimize the amount of time that the beads are exposed to air.
3	Break the vacuum immediately after each solution has been completely filtered from all wells (approximately 8-10 seconds). **Note** Wells typically filter at different rates.
4	Place the Filter Plate back on the Filter Plate .
5	Following the last wash in each series, blot the bottom of the Filter Plate thoroughly with a paper towel to remove traces of 1X Wash Buffer. Avoid touching the bottom of the Filter Plate with your fingers or to the bench during manipulations and immediately move to the next step to ensure that the beads are in the appropriate buffered solution.

Assay Preparation

About Preparing Samples

This instruction manual is specifically intended for the application of measuring cytokines from **Tissue or Cell Lysates**. We provide application specific manuals for measuring cytokines from Serum, Plasma and Cell Cultured Supernatants. You can visit our website to download our latest manuals.

Examples are provided below for measuring cytokines derived from brain and cultured cells. Other tissues can be used as a source of starting material, but these protocols have been provided as examples. For other tissue specific protocols, please call or email our technical support staff.

Preparing Brain Tissue Lysates

Brain Tissue Lysates

Step	Action
1	Animals are rapidly transcardially perfused with ice cold saline containing heparin to remove the blood.
2	The animal is then decapitated, the brain removed rapidly and 8mm carotid slices (or any size designed) cut and put into ice cold PBS
3	Regions of interest are excised from the slices. These tissue chunks are approx 2x2x8mm. The bigger of tissue size, the better, as long as same size for every sample, including treated and untreated. The size can go up to 10mg
4	The tissue chunks are immediately put into ice cold Procarta lysis buffer which contain protease inhibitors (150 µl per tissue chunk; we keep each chunk separate), and left on ice until all regions of interest are excised. 150 µL of Procarta Lysis Buffer can be used for up to 10 mg of tissue.
5	The tissue is then homogenized in a dounce homogenizer until there are no more tissue clumps (approx. 30- 40 strokes)
6	Centrifuge at 14,000 rpm (bench top centrifuge) for 10 minutes at 4° C.
7	The supernatant is transferred to a new tube and is ready for use. Store the samples at -80°C if they are to be used at a later date.

Preparing Lysates from Cultured Cells

Cell Culture Lysates

Step	Action
1	Adherent Cells Preparation is for 1x10^6 to 1x10^7 cells grown in a 10 cm plate. Trypinsinze the cells and remove the cells using a cell scraper. Transfer the cells to a 15 ml conical tube and centrifuge at 500 x g at 4° C for five minutes. Carefully aspirate the supernatant and re-suspend the cells in 5 mls of ice cold PBS. Repeat this step using 5 mls of ice cold PBS and centrifuge again at 500 x g at 4° C. Completely remove all of the PBS. Suspension Cells Preparation is for 1x10^6 to 1x10^7 cells grown in a flask. Transfer cells to a 15 ml conical tube and spin at 500 x g at 4° C for five minutes. Carefully aspirate the supernatant and re-suspend the cells in 5 mls of ice cold PBS and spin again at 500 x g at 4° C. Completely remove all of the PBS.
2	Add 200 µL of ice cold Procarta Cell Lysis Buffer, pipette up and down several times and then incubate on ice for 5 minutes.
3	Transfer entire contents to a 1.5 ml microcentrifuge tube

Cell Culture Lysates

Step	Action
4	Centrifuge at 14,000 rpm (bench top centrifuge) for 10 minutes at 4° C.
5	The supernatant is transferred to a new tube and is ready for use. Store the samples at -80°C if they are to be used at a later date.

Preparing Standards

To prepare standards:

Step	Action
1	Reconstitute the premixed lyophilized protein standard with 250 µL of Procarta Lysis Buffer
2	Vortex gently for 10 seconds and incubate on ice for 5 minutes. Transfer the entire contents to the first tube of the PCR 8-tube strip provided.
3	Prepare serial dilutions of the premixed standard as indicated below. a. Using the 8 tube strip provided, add the following b. Add 75 µL of Procarta Lysis Buffer to tubes 2-8. Follow the table below to create the dilutions. Use a P200 pipette to transfer and mix the 25 µL standard from tube to tube. c. Store tube strip on ice until ready to use. When transferring the standards to the to beads in the filter plate, we recommend using a multi-channel pipette. d. See Plate Map for example serial dilutions and blank

Tube Number	Procarta Lysis Buffer (µL)	Antigen Standard (µL)	Final Analyte Concentration in the Assay (pg/mL)
1	—	250	10,000
2	75	25 from Tube 1	2500
3	75	25 from Tube 2	625
4	75	25 from Tube 3	156
5	75	25 from Tube 4	39
6	75	25 from Tube 5	9.76
7	75	25 from Tube 6	2.4
8	75	25 from Tube 7	0.6

Blank	100 per well	Procarta Lysis Buffer	0

Preparing 1X Wash Buffer

Step	Action
1	Vortex the 10X Wash Buffer to dissolve all the salts.
2	Mix 20 mL of the 10X Wash Buffer with 180 mL deionized, sterile water. **Note** 1X Wash Buffer can be stored at 4 °C or room temperature for up to 6 months.

Assay Procedure

Assay Guidelines

IMPORTANT For optimal assay performance and consistent results, please read these guidelines before proceeding with the assay.

- Change the pipet tips after every transfer and avoid creating bubbles when pipetting.
- Follow the guidelines in "Set-Up and Operation of the Vacuum Manifold System" on page 8.
- During the incubation steps, cover the Filter Plate assembly with aluminum foil to prevent photobleaching of the fluorescent beads.
- Bring all buffers to room temperature before setting up the assay. Store the detection antibody, antibody beads, Streptavidin-PE, and reconstituted standards on ice during the assay.

Before You Start

Make sure the Luminex or Luminex-based instrument is turned on at least 30 minutes before you intend to read your plates.

Performing the Assay

To perform the assay:

Step	Action
1	Columns 1 and 2 are typically used for duplicate standards. Mark the sample wells you want to use. A blank plate layout is provided in the appendix for recording your plate layout.
2	Seal the un-used wells of the plate with a Plate Seal provided in the kit before starting the assay.
3	Pre-wet the Filter Plate: a. Add 150 µL of Reading Buffer to each non-sealed well. b. Incubate 5 minutes at room temperature. c. Remove the Reading Buffer with vacuum filtration. Reading Buffer should clear wells within 3–5 seconds. If outside this range, see "Set-Up and Operation of the Vacuum Manifold System" on page 8. **IMPORTANT** Do not invert the plate.
4	Add the Antibody Beads: a. Vortex the premixed Antibody Beads for 30 seconds at room temperature. b. Add 50 µL of Antibody Beads to each unsealed well. c. Remove buffer with vacuum filtration. **IMPORTANT** Do not invert the plate.
5	Wash the Antibody Beads: a. Add 150 µL/well of 1X Wash Buffer and remove with vacuum filtration. **IMPORTANT** Do not invert the plate. b. Blot the bottom of the Filter Plate thoroughly with paper towels to remove residual buffer.
6	Add 75 µL/well of Procarta Lysis Buffer to only the Standard Wells.

To perform the assay: *(continued)*

Step	Action
7	Add 25 µL/well of standards marked on the plate layout sheet. For the sample, add 100 µL/well of Lysate. For the blank samples, add 100 µL/well of Procarta Lysis Buffer. Seal the plate gently using the Plate Seal. If you are using 25 µL or 50 uL of Lysate, you will need to add 75 or 50 uL of Procarta Lysis Buffer for a total of 100 µL for each sample well.
8	Incubate and wash the Filter Plate: a. Completely wrap the Filter Plate with aluminum foil. b. Shake for 30 minutes at 500 rpm at room temperature. Optionally, you can incubate overnight without shaking at 4 °C, after the 30 minute shaking incubation step. c. Carefully remove the Plate Seal to avoid splashing the plate contents. d. Remove solution with vacuum filtration. **IMPORTANT** Do not invert the plate. e. When washing the plate, we recommend using multi-channel pipette and a large enough plastic reservoir for the wash buffer. We also recommend stationing the wash buffer reservoir next manifold to minimize the amount of time that the beads are exposed to air. f. Wash the plate three times with 150 µL/well of 1X Wash Buffer. After the 3rd wash, blot the bottom of the Filter Plate with paper towels.
9	Add premixed Detection Antibodies: a. Add 25 µL/well of the Detection Antibody. b. Seal the Filter Plate with a new Plate Seal. c. Completely wrap the Filter Plate with aluminum foil. d. Shake for 30 minutes at 500 rpm at room temperature.
10	Remove the solution with vacuum filtration and wash the plate: a. Carefully remove the Plate Seal to avoid splashing the plate contents. b. Remove solution with vacuum filtration. **IMPORTANT** Do not invert the plate. c. Wash the plate three times with 150 µL/well of 1X Wash Buffer. After the 3rd wash, blot the bottom of the Filter Plate with paper towels.
11	Add Streptavidin–PE: a. Vortex the Streptavidin–PE. b. Add 50 µL/well of Streptavidin-PE. c. Seal the Filter Plate with a new Plate Seal. d. Completely wrap the Filter Plate with aluminum foil. e. Shake for 30 minutes at 500 rpm at room temperature.
12	Remove the solution with vacuum filtration and wash the plate: a. Carefully remove the Plate Seal to avoid splashing the plate contents. b. Remove solution with vacuum filtration. **IMPORTANT** Do not invert the plate. c. Wash the plate three times with 150 µL/well of 1X Wash Buffer. After the 3rd wash, blot the bottom of the Filter Plate with paper towels.

To perform the assay: *(continued)*

Step	Action
13	Prepare plate for analysis on a Luminex instrument: a. Add 120 µL/well of the Reading buffer. b. Seal the Filter Plate with a new Plate Seal. **Note** The Filter Plate can be wrapped with aluminum foil and stored for up to 48 hours before proceeding. However, delay in reading the plate may result in decreased sensitivity for some analytes. c. Shake for 5 minutes at 500 rpm at room temperature or until the Luminex instrument is available. d. Remove the Plate Seal before analyzing on the Luminex instrument.

To perform the assay: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>14</td><td>Analyze the plate following the respective operation manual for the Luminex or Luminex-based
instrument.

<table>
<tr><th>Software</th><th>Sample Size</th><th>DD Gate</th><th>Timeout</th><th>Bead Events/Bead Region</th></tr>
<tr><td>Luminex 100/
IS100 v 2.318</td><td>50 µL</td><td>7,500-15,000</td><td>25 sec</td><td>50 or 100</td></tr>
<tr><td>Bioplex/
Bio-Rad</td><td>50 µL</td><td>4335-10,000</td><td>45 sec</td><td>50 or 100</td></tr>
<tr><td>Miraibio/
Hitachi</td><td>50 µL</td><td>2,000-15,000</td><td>25 sec</td><td>50 or 100</td></tr>
<tr><td>Starstation/
ACS</td><td>50 µL</td><td>4,335-10,000</td><td>25 sec</td><td>50 or 100</td></tr>
</table>

When the Luminex beads are injected into the flow cell, a small percentage of the beads will have a tendency to clump and go through the flow cell as doublets. The DD Gate or Doublet Discriminator Gate will allow for discrimination of the doublets from the singlet beads. When setting the DD Gate, you can follow the appropriate settings from the table above. However, for the best results, you should adjust the DD Gate and center the gates around the largest peak which is the singlet beads. You can adjust the gates when processing the first sample.

The Bio-Plex® Suspension Array System allows calibration using Low or High sensitivity settings. Perform the sensitivity selection during calibration, using predetermined values of CAL2 RP1 target, as provided by Bio-Rad. Using the RP1 Low target value will provide results comparable to those obtained from the Luminex 100. Using the RP1 High target value may increase detection sensitivity for low cytokine protein concentrations but sacrifices part of the linear dynamic range for high concentrations. We recommend RP1 Low target value for BioPlex.

The number of beads events per region can be set to 50 or 100. Please note that the total time to measure 100 events vs 50 events will take longer.

IMPORTANT Check to ensure the probe height in the Luminex instrument is adjusted appropriately for the Filter Plate.

IMPORTANT We recommend that you calibrate the Luminex or Luminex-based instrument each day the assay is run.

IMPORTANT If there is a malfunction of the machine or software during the run or there was a problem with the system, the plate can be re-processed on the Luminex machine. Remove the plate from the machine and vacuum filter the plate. Resuspend the beads in Reading Buffer and shake for 5 minutes then re-read. The plate may take longer to read since there will be less beads in the plate.</td></tr>
</table>

Troubleshooting

Possible Problems and Recommended Solutions

Observation	Possible Cause	Recommended Action
Filter plate leakage	Vacuum pressure too high	Adjust the vacuum pressure as recommended in "Set-Up and Operation of the Vacuum Manifold System" on page 8.
	Filter Plate is misaligned (at an angle) during incubation/processing	Set the Filter Plate on a flat, level surface during incubation/processing.
	Leakage from capillary action	After each vacuum step, blot the bottom of the Filter Plate using paper towels or absorbent paper.
	Plate Seal applied using too much force	Lay Plate Seal on the plate and gently roll a 5 mL serological pipet over the plate to seal.
High CV	Samples and antigen standards were not stored on ice	Prepare the samples and standards on ice before setting up the assay.
	Bottom of the Filter Plate is not dry	After each vacuum step, blot the bottom of the Filter Plate using paper towels or absorbent paper.
	Contamination from re-using the Plate Seal	Use a new Plate Seal for each incubation step.
	The high concentration standards have high variability.	After adding the standards and samples, it is very important that any excess standards are removed during the wash step
	Contamination from Wash Buffer	Be careful not to splash Wash Buffer during wash steps into adjacent wells.
Limited Dynamic Range when using the BioPlex software	The machine was calibrated using the high PMT (CAL2 High RP1) and the top 3 standards have similar MFI values	Calibrate the machine using the CAl2 Low RP1 target value

Observation	Possible Cause	Recommended Action
Low bead count	Volume of bead solution is too low	Add 120 µL/well Reading Buffer and shake for 5 minutes to resuspend beads before reading in the Luminex instrument.
	Beads are clumping	Vortex the bead solution well before using in the assay.
	Vacuum pressure too high	Use 2–3 mm Hg vacuum pressure.
	Filter ruptured due to excess vacuum time	Do not use the vacuum over 10 seconds in any of the steps.
	Dyes contained in the beads are photo-bleached from overexposure to light	Store bead solution and the assay plate in the dark
	Reader is clogged	Follow the instructions in the Luminex instrument user documentation.
Low signal or sensitivity	Standards not reconstituted and diluted correctly	Prepare fresh antigen standards following the instructions in “Preparing Standards” on page 11.
	Beads or reagents expired	Verify the expiration date of the kit.
	Beads stuck to the bottom of the plate	Make sure the plate is agitated at 500 rpm for the recommended time and for at least 5 minutes before reading.
Poor recovery	Reagents expired	Verify the expiration date on the kit.
	Samples and antigen standards were not stored on ice	Prepare the samples and standards on ice before setting up the assay.

Contacting Panomics

Technical Help

For technical questions, contact our technical support group by telephone at 866-317-2626 or by email at techsupport@panomics.com, or visit our website www.panomics.com for an updated list of FAQs and product support literature.

For Additional Services

For information about Panomics products or for ordering information, contact your Regional Sales Manager, or visit our website at www.panomics.com.

Appendix

Bead-Analyte Associations

The following table may not be the most up to date current bead-analyte associations setting for your Luminex instrument. Please refer to your product insert for analytes included in your kit, especially if you have a custom order or “By Request” assay.

Bead	Human	Bead	Human	Bead	Mouse	Bead	Rat
66	Adiponectin	22	IP10	24	Adiponectin	73	CRP
54	CRP	74	I-TAC	8	CD120B	52	Eotaxin
84	EGF	6	Leptin	52	Eotaxin	46	G-CSF
17	ENA78	51	MCP - 1	46	G-CSF	36	GM-CSF
52	Eotaxin	13	MCP - 3	44	GM-CSF	21	ICAM
55	FGF - basic	80	MIF	43	IFM-gamma	43	IFN-gamma
46	G-CSF	11	MIG	18	IL - 1 alpha	18	IL-1 alpha
44	GM-CSF	8	MMP- 1	19	IL - 1 beta	19	IL-1 beta
61	GRO alpha	7	MMP- 2	20	IL - 2	20	IL-2
79	HGF	53	MMP- 3	21	IL - 3	34	IL-4
73	ICAM - 1	47	MIP - 1 beta	25	IL - 4	27	IL-5
48	IFN alpha	67	MIP - 3 alpha	26	IL - 5	25	IL-6
43	IFNy	12	MIP - 1 alpha	27	IL - 6	35	IL-10
62	IL - 1 alpha	83	MMP - 7	38	IL - 9	32	IL-12(p40)
18	IL - 1 beta	72	MMP - 8	28	IL - 10	44	IL-13
63	IL - 1ra	9	MMP - 9	33	IL - 12(p40)	28	IL-17
19	IL - 2	78	MMP - 12	34	IL - 12(p70)	57	IL-18
20	IL - 4	64	MMP - 13	36	IL - 13	37	KC
21	IL - 5	38	NGF	35	IL - 17	6	Leptin
25	IL - 6	14	PAI - 1	41	IL-23	54	MCP - 1
26	IL - 7	37	PDGFBB	22	IP10	51	MCP - 3
27	IL - 8	42	RANTES	37	KC	47	MIP-1 alpha
28	IL - 10	86	Resistin	6	Leptin	38	NGF
33	IL - 12(p40)	75	SAA	54	MCP - 1	42	RANTES
34	IL - 12(p70)	85	sCD40L	51	MCP - 3	29	TGF beta
35	IL - 13	77	sE-selectin	47	MIP - 1 alpha	33	TNF alpha
65	IL - 15	29	TGF beta	42	RANTES	26	V-CAM
36	IL - 17	45	TNF alpha	7	Srankl	56	VEGF
39	IL - 17F	32	TNF beta	29	TNF beta		
57	IL-18	88	VCAM - 1	45	TNF alpha		
41	IL-23	56	VEGF	56	VEGF		

*TGF-beta must be used as a single plex assay because the sample must be prepared in an acidic buffer.

Assay Performance

Standards Comparison

The following table contains conversion factors for Procarta Antigen Standards for which World Health Organization (WHO) standards were available for comparison.

Procarta Antigen Standards, Human

Analyte	WHO Lot Number	100 Procarta pg/mL =
IL-1β	86/680	11.9 IU/mL
IL-4	88/656	15.6 IU/mL
IL-8	89/520	1.91 IU/mL
IL-10	93/722	3.91 IU/mL
IL-12(p70)	95/544	11.9 IU/mL
IL-13	94/622	1.33 IU/mL
IFNγ	88/606	29.4 IU/mL
GM-CSF	88/646	20.3 IU/mL
TNFα	88/786	147.7 IU/mL

Procarta Antigen Standards, Mouse

Analyte	WHO Lot Number	100 Procarta pg/mL =
IL-1α	93/672	580.8 IU/mL
IL-1β	93/668	99.8 IU/mL
IL-2	93/566	67.3 IU/mL
IL-3	91/662	141.0 IU/mL
IL-4	91/656	8.2 IU/mL
IL-6	93/730	97.6 IU/mL
GM-CSF	88/532	151.5 IU/mL
TNFα	91/658	92.5 IU/mL

Limit of Detection

The Limit of Detection (LOD) for human, mouse and rat cytokines from cell culture supernatants, serum, and plasma samples is less than 1 pg/ml. The LOD is defined as the antigen concentration with a Median Fluorescent Intensity (MFI) greater than or equal to the sum of the background MFI plus two standard deviation

Assay Cross Reactivity and Specificity

Antibody cross reactivity is negligible and does not exceed 5%. Cross reactivity was verified by examining the non-specific signal from each bead after:

- A single antigen was added
- All antigens, except the antigen matching the bead being evaluated, was added

Assay Precision

The following tables present typical intra-assay and inter-assay coefficients of variation (CVs) for human samples from cell culture supernatants, plasma, and serum. The average intra-assay and inter-assay CV is less than 10% based on the analysis of 4 replicates.

Assay Accuracy

Accuracy is defined as the percentage of analyte recovered from samples spiked with a known quantity. The following table represents typical spike recovery results based on spiking 3 different amounts (40, 400, and 4000 pg/mL) of analyte into cell-culture supernatants, serum, and plasma samples from human and mouse. All samples had low or undetectable amount of analyte present before the spike. Each sample type had 4 replicates.

The spike recovery was 80–120%. The percent spike recovery is defined as the observed Median Fluorescent Intensity (MFI) divided by the expected MFI times 100.

Sample and Blank Plate Layouts

	Standards		Samples									
	1 pg/mL	**2 pg/mL**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	20,000	20,000	Blank	Blank	8	8	16	16	24	24	32	32
B	5,000	5,000	1	1	9	9	17	17	25	25	33	33
C	1250	1250	2	2	10	10	18	18	26	26	34	34
D	313	313	3	3	11	11	19	19	27	27	35	35
E	78	78	4	4	12	12	20	20	28	28	36	36
F	19.5	19.5	5	5	13	13	21	21	29	29	37	37
G	4.9	4.9	6	6	14	14	22	22	30	30	38	38
H	1.2	1.2	7	7	15	15	23	23	31	31	39	39