Mosaic™ ELISA

Human Cytokine Panel 1

Catalog Number MEA001

For the simultaneous quantitative determination of concentrations of multiple human cytokines in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Cytokines are pleiotropic extracellular signaling molecules that influence myriad physiological and pathological processes. Released from a variety of cells, their actions affect cellular growth, differentiation, gene expression, migration, immunity, and inflammation. In most biological processes, multiple cytokines operate in a large network where the action of one cytokine is regulated by the presence or absence of other cytokines. Studying the underlying signaling cascades is further complicated by the fact that many cytokines modulate the production of other cytokines. The Mosaic™ Human Cytokine Panel 1 is an excellent tool for the detection of 8 different cytokines in the same sample. Mosaic kits employ multiplex microarray technology to provide an accurate, efficient, and economical alternative to conducting multiple traditional ELISA experiments.

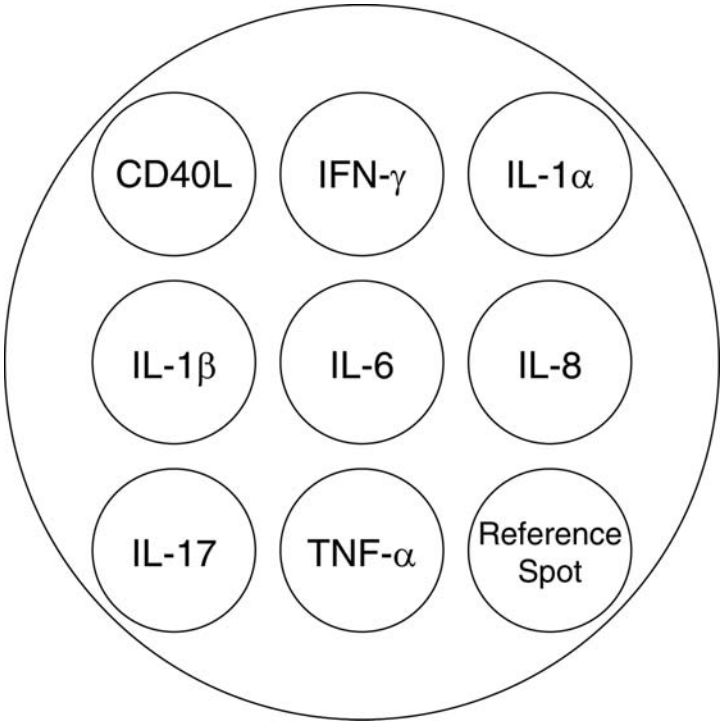


Figure 1: A visualization of the spot layout per well.

PRINCIPLE OF THE ASSAY

The Mosaic Human Cytokine Panel 1 Immunoassay employs a two-site sandwich ELISA technique to simultaneously detect 8 cytokines in cell culture supernates, serum, and plasma. Multiple capture antibodies that specifically recognize the target cytokines have been pre-spotted into each well of a 96 well microplate. Standards and samples are added, and cytokines present in the samples are bound by the immobilized antibodies. After washing away unbound material, biotinylated detection antibodies are used to detect the specific cytokines. Unbound detection antibodies are washed away and streptavidin-HRP is added. Following an additional wash, chemiluminescent substrate reagents are added to the wells, and a signal proportional to the amount of each cytokine bound in the initial step is produced. Plates are read using a digital camera imaging system, and pixel intensity is measured using an analytical software package.

TECHNICAL HINTS AND LIMITATIONS

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in buffers, operator, pipetting technique, washing technique, instrumentation, and incubation time or temperature and kit age can alter the performance of the kit.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Avoid microbial contamination of reagents and buffers.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Mosaic affords the user the benefit of multianalyte analysis of 8 cytokines in a complex sample. A multipurpose diluent for each sample type is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- This assay is designed to eliminate interference by receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Mosaic assay, the possibility of interference cannot be excluded.
- Discrepancies may exist in values obtained for the same analyte utilizing different technologies.
- **Only the analytes listed in Figure 1 (or on the enclosed Standard Value Card) can be measured with this kit.**

MATERIALS PROVIDED

Microplate (Part 893391) - 96 well microplate spotted with 8 antibodies against specific cytokines.

Standard (Part 893392) - 2 vials of a cocktail of recombinant human cytokines in a buffered protein base with preservatives; lyophilized.

Standard Value Card (Part 749064) - 1 card listing the standard reconstitution volume and concentrations for this lot of standard.

Detection Mix (Part 895470) - 6 mL/vial of a cocktail of antibodies conjugated to biotin with preservatives.

Assay Diluent RD1-102 (Part 895939) - 6 mL/vial of a buffered protein base with blue dye and preservatives.

Calibrator Diluent RD5K (Part 895119) - 21 mL of buffered protein base with preservatives (*for cell culture supernate samples*).

Calibrator Diluent RD6-40 (Part 895817) - 21 mL of a buffered protein base with preservatives (*for serum/plasma samples*). *May contain a precipitate. Mix well before and during use.*

Wash Buffer Concentrate (Part 895003) - 2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative.

Streptavidin-HRP (Part 895469) - 6 mL/vial of a streptavidin-horseradish peroxidase conjugate with preservatives.

Substrate 1 (Part 895471) - 3 mL/vial of a buffered solution.

Substrate 2 (Part 895472) - 3 mL/vial of a buffered solution.

Plate Sealers (Part 640197) - 8 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Calibrator Diluent RD5K</td></tr>
<tr><td>Calibrator Diluent RD6-40</td></tr>
<tr><td>Assay Diluent RD1-102</td></tr>
<tr><td>Streptavidin-HRP</td></tr>
<tr><td>Unmixed Substrate 1</td></tr>
<tr><td>Unmixed Substrate 2</td></tr>
<tr><td>Detection Mix</td></tr>
<tr><td>Standard</td><td>Discard after use. Use a fresh standard for each assay.</td></tr>
<tr><td>Microplate</td><td>Invert the plate, and blot it against clean paper towels to dry the plate. Return it to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, squirt bottle, or automated microplate washer.
- Graduated cylinders for preparing Wash Buffer.
- Horizontal orbital microplate shaker (0.12” orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Digital Imaging System (for details, visit www.RnDSystems.com/go/ImagingSystems).
- **Polypropylene** test tubes for dilution.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay. Grossly hemolyzed samples are not suitable for use in this assay.*

Platelet-Poor Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2 - 8° C at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

CD40 Ligand is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of CD40 Ligand, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

SAMPLE PREPARATION

Serum/plasma/platelet-poor plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 60 µL of sample + 60 µL of Calibrator Diluent RD6-40. Mix thoroughly.

Cell culture supernate samples may require dilution.

REAGENT PREPARATION

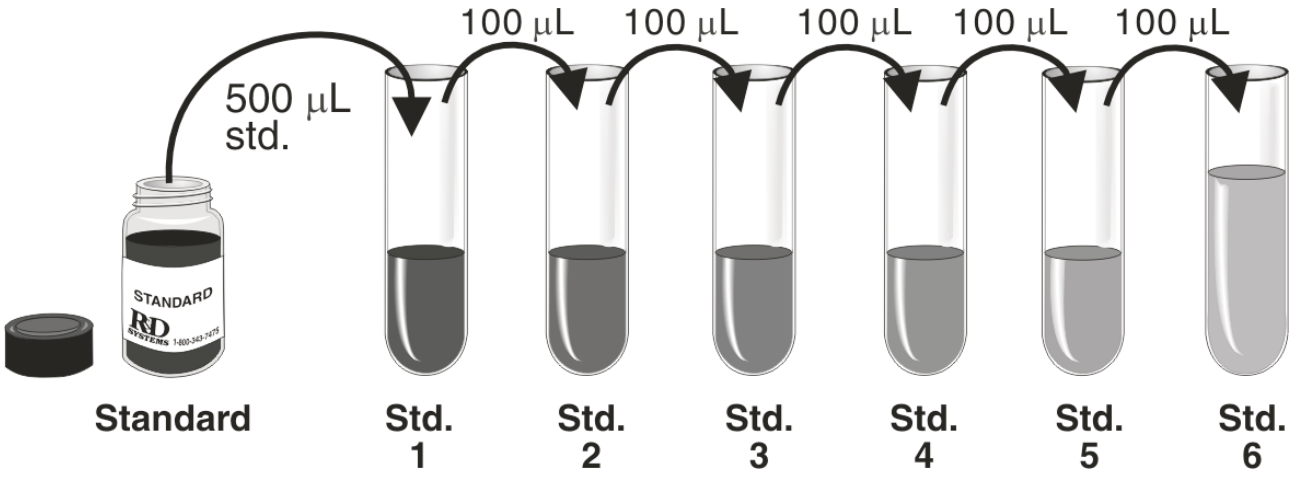
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Substrates 1 and 2 should be mixed together in equal volumes 2 - 30 minutes prior to use. Protect from light. 50 µL of the resultant mixture is required per well.

Standard - Reconstitute the Standard Cocktail with Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6-40 (*for serum/plasma samples*). Refer to the Standard Value Card for the reconstitution volume. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 500 µL of the reconstituted Standard into the Standard 1 tube. Pipette 200 µL of the appropriate Calibrator Diluent into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: *Protect Streptavidin-HRP and the Substrate from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 50 µL of Assay Diluent RD1-102 to each well.
3. Add 50 µL of Standard or sample* per well. Securely cover with a plate sealer. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 50 µL of the Detection Mix to all wells. Securely cover with a plate sealer and incubate for 2 hours at room temperature on the shaker set at 500 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of Streptavidin-HRP to all wells. Securely cover with a plate sealer and incubate for 30 minutes at room temperature on the shaker set at 500 ± 50 rpm.
8. Aspirate each well and wash as in step 4, repeating the process five times for a total of six washes.
9. Add 50 µL of Substrate Solution to each well.
10. Place the microplate in the imager. Wait no longer than 15 minutes to commence imaging.

    Note: *For details, visit www.RnDSystems.com/go/ImagingSystems.*

*Samples may require dilution. See the Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.

2. Add 50 µL of Assay Diluent RD1-102 to each well.

3. Add 50 µL of Standard or sample* to each well. Incubate for 2 hours at RT on a horizontal orbital microplate shaker.

4. Aspirate and wash 4 times.

5. Add 50 µL of Detection Mix to all wells. Incubate for 2 hours at RT on the shaker.

6. Aspirate and wash 4 times.

7. Add 50 µL of Streptavidin-HRP to all wells. Incubate for 30 minutes at RT on the shaker.

8. Aspirate and wash 6 times.

9. Add 50 µL of Substrate Solution to each well.

10. Read within 15 minutes of Substrate addition.

Protect the Streptavidin-HRP and Substrate from light at all times.

*Samples may require dilution. See the Sample Preparation section.

INSTRUMENTATION

The Mosaic ELISA Kits have been validated on the Q-View™ Imager from Quansys Biosciences. Please visit **www.RnDSystems.com/go/ImagingSystems** for suitable imaging systems and their instructions for use.

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) was determined by adding two standard deviations to the mean pixel intensity of twenty zero standard replicates and calculating the corresponding concentration.

Analyte	Mean (pg/mL)	Range (pg/mL)
CD40 Ligand	2.23	1.43 - 5.31
IFN-γ	1.38	0.77 - 2.08
IL-1α	0.59	0.33 - 0.74
IL-1β	0.20	0.11 - 0.31
IL-6	0.41	0.21 - 0.76
IL-8	0.59	0.26 - 1.24
IL-17	0.17	0.06 - 0.35
TNF-α	1.65	0.76 - 2.13

CALIBRATION

This assay is calibrated against highly purified recombinant human cytokines produced at R&D Systems.

Q-View is a trademark of Quansys Biosciences.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average zero standard median pixel intensity (PI).

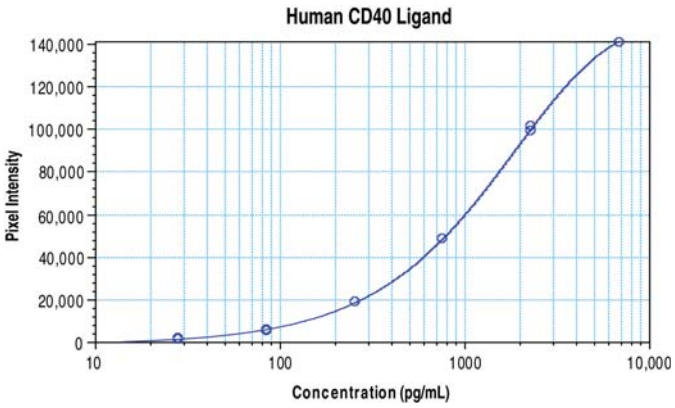
Create a standard curve for each analyte by reducing the data using computer software capable of generating a 5-PL curve fit. As an alternative, construct a standard curve by plotting the median PI for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the concentrations versus the log of the PI and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

To determine the concentration of each sample, first find the PI value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration.

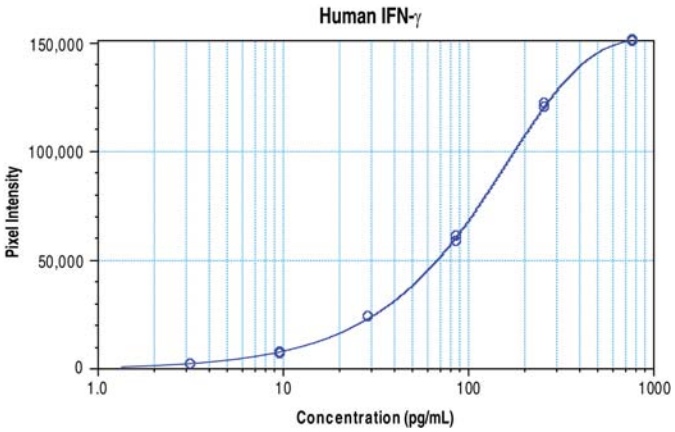
Since serum and plasma samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

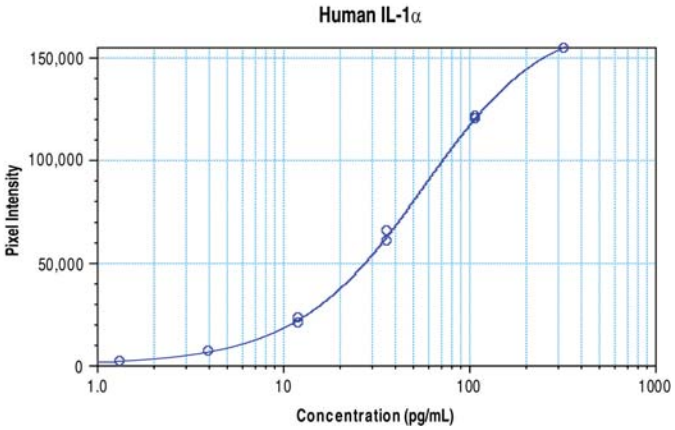
These standard curves are provided only for demonstration. Standard curves must be generated each time an assay is run, utilizing values from the included Standard Value Card.



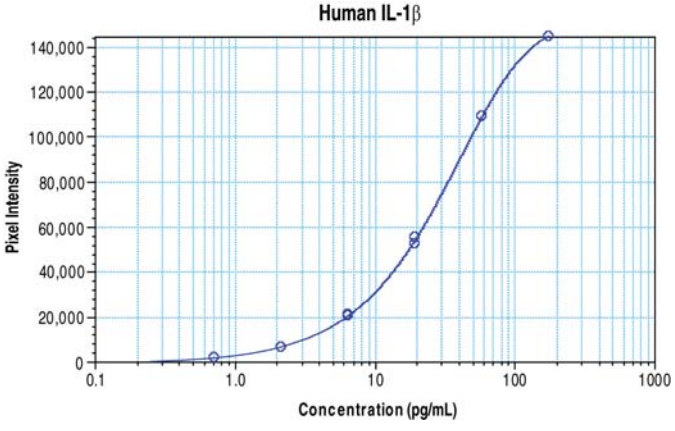
Standard	pg/mL	PI	Average	Corrected
Blank	0	1356 1402	1379	—
Std 1	6900	142,087 142,920	142,504	141,125
Std 2	2300	100,229 102,704	101,467	100,088
Std 3	767	49,617 49,982	49,800	48,421
Std 4	256	19,889 20,098	19,994	18,615
Std 5	85	6660 6820	6740	5361
Std 6	28	2801 2848	2825	1446



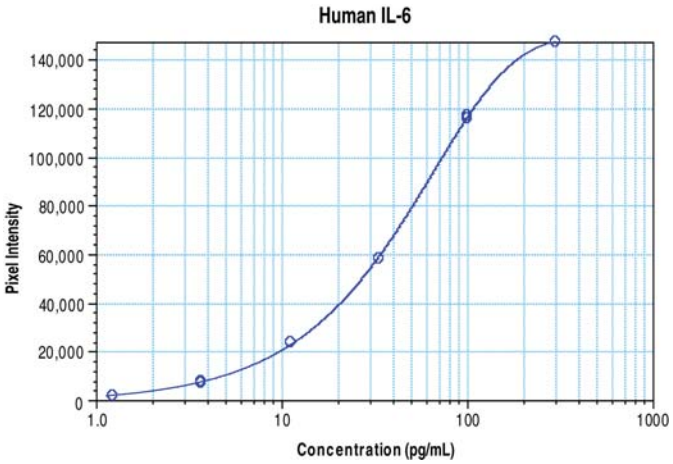
Standard	pg/mL	PI	Average	Corrected
Blank	0	1154 1252	1203	—
Std 1	780	151,778 152,279	152,029	150,826
Std 2	260	121,408 122,899	122,154	120,951
Std 3	87	59,882 62,220	61,051	59,848
Std 4	29	25,060 25,245	25,153	23,950
Std 5	10	8140 8510	8325	7122
Std 6	3.2	3109 3301	3205	2002



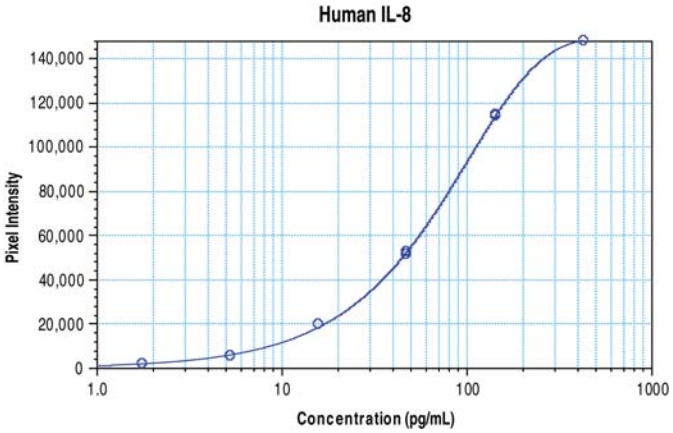
Standard	pg/mL	PI	Average	Corrected
Blank	0	1305 1350	1328	—
Std 1	325	155,588 157,541	156,565	155,237
Std 2	108	121,487 122,500	121,994	120,666
Std 3	36	61,822 66,785	64,304	62,976
Std 4	12	21,848 24,639	23,244	21,916
Std 5	4.0	7942 8515	8229	6901
Std 6	1.3	2925 3346	3136	1808



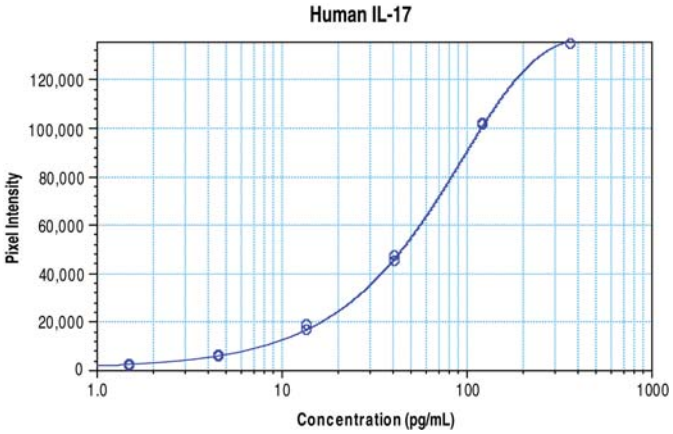
Standard	pg/mL	PI	Average	Corrected
Blank	0	1187 1190	1189	—
Std 1	175	145,662 146,090	145,876	144,687
Std 2	58	109,897 109,980	109,939	108,750
Std 3	19	53,691 56,789	55,240	54,051
Std 4	6.5	21,359 22,277	21,818	20,629
Std 5	2.2	7413 7572	7493	6304
Std 6	0.7	2724 3223	2974	1785



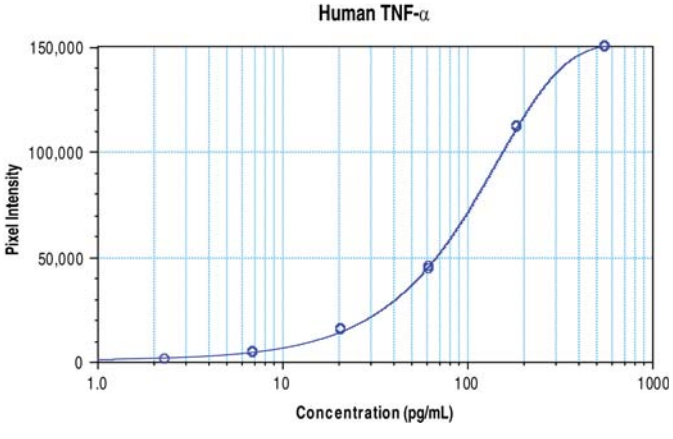
Standard	pg/mL	PI	Average	Corrected
Blank	0	1231 1317	1274	—
Std 1	300	148,245 148,889	148,567	147,293
Std 2	100	117,233 118,408	117,821	116,547
Std 3	33	59,620 59,686	59,653	58,379
Std 4	11	24,722 24,783	24,753	23,479
Std 5	3.7	8357 8935	8646	7372
Std 6	1.2	2971 3150	3061	1787



Standard	pg/mL	PI	Average	Corrected
Blank	0	1424 1444	1434	—
Std 1	430	148,960 149,650	149,305	147,871
Std 2	143	115,377 115,899	115,638	114,204
Std 3	48	52,811 53,702	53,257	51,823
Std 4	16	21,064 21,075	21,070	19,636
Std 5	5.3	6786 6919	6853	5419
Std 6	1.8	2938 3007	2973	1539



Standard	pg/mL	PI	Average	Corrected
Blank	0	1604 1610	1607	—
Std 1	370	136,206 138,488	137,347	135,740
Std 2	123	102,622 103,357	102,990	101,383
Std 3	41	46,252 48,418	47,335	45,728
Std 4	14	18,133 19,945	19,039	17,432
Std 5	4.6	7237 7724	7481	5874
Std 6	1.5	3513 3796	3655	2048



Standard	pg/mL	PI	Average	Corrected
Blank	0	1243 1272	1258	—
Std 1	560	151,019 152,027	151,523	150,265
Std 2	187	113,161 113,598	113,380	112,122
Std 3	62	45,777 46,970	46,374	45,116
Std 4	21	16,135 16,773	16,454	15,196
Std 5	6.9	5339 5970	5655	4397
Std 6	2.3	2503 2560	2532	1274

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in thirty separate assays to assess inter-assay precision.

CD40 Ligand Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	236	1103	6437	222	991	6310
Standard deviation	12.8	38.0	401.3	25.7	79.2	421.3
CV (%)	4.2	2.7	4.9	11.5	8.0	6.7

IFN-γ Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	83	382	1786	68	325	1763
Standard deviation	5.5	14.1	99.9	8.1	24.9	103.2
CV (%)	5.4	3.0	4.6	11.9	7.7	5.9

IL-1α Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	34	136	689	29	127	732
Standard deviation	1.8	7.0	43.8	3.2	7.1	34.5
CV (%)	4.6	4.4	5.4	10.9	5.6	4.7

IL-1β Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	16	68	371	16	65	372
Standard deviation	0.8	2.9	24.7	1.8	4.2	21.1
CV (%)	4.3	3.9	6.1	11.3	6.5	5.7

IL-6 Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	43	178	843	38	161	910
Standard deviation	2.3	8.8	93.6	3.6	10.5	79.6
CV (%)	4.8	4.3	9.8	9.5	6.5	8.8

IL-8 Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	52	205	963	41	170	970
Standard deviation	4.9	10.5	85.9	5.1	12.6	65.2
CV (%)	7.9	4.3	7.5	12.3	7.4	6.7

IL-17 Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	19	81	394	15	66	408
Standard deviation	1.1	2.4	21.3	2.0	4.8	30.0
CV (%)	5.3	2.8	5.2	13.0	7.2	7.3

TNF-α Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	30	30	30
Mean (pg/mL)	70	355	1720	57	270	1675
Standard deviation	7.1	13.2	83.5	7.2	22.6	96.9
CV (%)	8.2	3.0	3.9	12.6	8.4	5.8

RECOVERY

The recovery of cytokines spiked to levels throughout the range of the assay in various matrices was evaluated.

CD40 Ligand Assay

Sample	Average % Recovery	Range
Cell culture supernates	93	82 - 102%
Serum	101	90 - 117%
EDTA plasma	93	86 - 105%
Heparin plasma	92	81 - 115%
Platelet-poor EDTA plasma	93	85 - 101%
Platelet-poor Heparin plasma	91	80 - 102%

IFN-γ Assay

Sample	Average % Recovery	Range
Cell culture supernates	94	70 - 110%
Serum	91	84 - 103%
EDTA plasma	91	65 - 109%
Heparin plasma	80	75 - 102%
Platelet-poor EDTA plasma	86	74 - 97%
Platelet-poor Heparin plasma	76	67 - 83%

IL-1α Assay

Sample	Average % Recovery	Range
Cell culture supernates	93	79 - 105%
Serum	92	74 - 119%
EDTA plasma	93	85 - 111%
Heparin plasma	94	85 - 112%
Platelet-poor EDTA plasma	94	84 - 111%
Platelet-poor Heparin plasma	90	64 - 105%

IL-1β Assay

Sample	Average % Recovery	Range
Cell culture supernates	98	90 - 108%
Serum	98	70 - 110%
EDTA plasma	96	68 - 110%
Heparin plasma	94	73 - 109%
Platelet-poor EDTA plasma	97	86 - 102%
Platelet-poor Heparin plasma	93	83 - 100%

IL-6 Assay

Sample	Average % Recovery	Range
Cell culture supernates	92	80 - 110%
Serum	97	90 - 105%
EDTA plasma	96	86 - 106%
Heparin plasma	93	75 - 108%
Platelet-poor EDTA plasma	91	84 - 99%
Platelet-poor Heparin plasma	87	80 - 95%

IL-8 Assay

Sample	Average % Recovery	Range
Cell culture supernates	105	92 - 113%
Serum	98	85 - 114%
EDTA plasma	92	83 - 100%
Heparin plasma	93	77 - 109%
Platelet-poor EDTA plasma	90	81 - 97%
Platelet-poor Heparin plasma	89	80 - 101%

IL-17 Assay

Sample	Average % Recovery	Range
Cell culture supernates	106	95 - 116%
Serum	105	71 - 131%
EDTA plasma	102	77 - 139%
Heparin plasma	92	68 - 127%
Platelet-poor EDTA plasma	95	77 - 105%
Platelet-poor Heparin plasma	89	71 - 103%

TNF-α Assay

Sample	Average % Recovery	Range
Cell culture supernates	103	89 - 137%
Serum	90	77 - 118%
EDTA plasma	86	64 - 108%
Heparin plasma	85	67 - 134%
Platelet-poor EDTA plasma	84	74 - 91%
Platelet-poor Heparin plasma	80	65 - 88%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of cytokines were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

CD40 Ligand Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	101	104	106	109	97	98
	Range (%)	98 - 105	100 - 109	100 - 110	101 - 118	92 - 99	95 - 102
1:4	Average % of Expected	100	101	101	107	98	99
	Range (%)	95 - 106	94 - 105	88 - 108	94 - 118	94 - 100	97 - 102
1:8	Average % of Expected	100	103	100	102	101	100
	Range (%)	92 - 106	95 - 110	84 - 107	85 - 115	99 - 102	98 - 102

IFN-γ Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	99	101	102	108	99	104
	Range (%)	96 - 101	100 - 102	100 - 103	105 - 111	96 - 102	103 - 106
1:4	Average % of Expected	98	102	103	111	102	108
	Range (%)	96 - 101	100 - 103	101 - 104	100 - 119	99 - 105	102 - 112
1:8	Average % of Expected	100	103	102	109	103	110
	Range (%)	92 - 108	101 - 105	100 - 105	95 - 115	99 - 107	106 - 114

IL-1α Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	102	107	103	105	99	101
	Range (%)	99 - 104	100 - 122	100 - 105	103 - 109	95 - 102	96 - 103
1:4	Average % of Expected	95	106	103	105	98	100
	Range (%)	91 - 102	100 - 120	100 - 105	101 - 109	96 - 102	97 - 103
1:8	Average % of Expected	94	102	102	100	98	99
	Range (%)	84 - 101	92 - 116	98 - 110	95 - 106	87 - 103	97 - 100

IL-1β Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	100	95	96	100	96	101
	Range (%)	95 - 107	85 - 100	91 - 99	93 - 107	95 - 98	97 - 106
1:4	Average % of Expected	103	102	103	107	98	102
	Range (%)	100 - 106	97 - 106	94 - 107	92 - 118	97 - 98	98 - 105
1:8	Average % of Expected	109	106	105	104	99	100
	Range (%)	103 - 116	102 - 108	97 - 110	91 - 111	96 - 100	98 - 103

IL-6 Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	104	108	106	114	100	105
	Range (%)	101 - 107	103 - 111	97 - 113	105 - 126	98 - 105	101 - 107
1:4	Average % of Expected	100	106	103	113	103	107
	Range (%)	95 - 104	100 - 110	92 - 111	104 - 124	99 - 112	102 - 110
1:8	Average % of Expected	100	106	103	108	106	109
	Range (%)	97 - 106	101 - 108	93 - 111	100 - 120	102 - 117	103 - 114

IL-8 Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	101	108	105	110	102	104
	Range (%)	98 - 104	105 - 113	102 - 107	104 - 118	98 - 106	101 - 107
1:4	Average % of Expected	94	107	107	110	104	106
	Range (%)	89 - 99	101 - 112	102 - 111	102 - 122	100 - 106	102 - 112
1:8	Average % of Expected	95	111	107	111	115	117
	Range (%)	93 - 96	104 - 116	102 - 113	105 - 124	108 - 119	111 - 121

IL-17 Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	107	107	106	105	96	99
	Range (%)	105 - 110	90 - 117	98 - 119	88 - 119	95 - 97	93 - 104
1:4	Average % of Expected	102	109	102	110	98	100
	Range (%)	89 - 114	91 - 125	88 - 114	87 - 120	96 - 99	93 - 105
1:8	Average % of Expected	93	113	107	111	96	99
	Range (%)	77 - 114	87 - 134	89 - 124	96 - 121	92 - 103	90 - 107

TNF-α Assay

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Platelet-Poor	
						EDTA plasma	Heparin plasma
1:2	Average % of Expected	102	106	103	105	108	106
	Range (%)	97 - 107	99 - 113	98 - 111	96 - 112	105 - 111	98 - 112
1:4	Average % of Expected	105	110	104	110	117	112
	Range (%)	94 - 115	98 - 122	97 - 119	94 - 124	108 - 123	98 - 122
1:8	Average % of Expected	106	115	103	111	126	117
	Range (%)	98 - 123	100 - 130	84 - 124	88 - 126	115 - 132	101 - 130

SAMPLE VALUES

Serum/Plasma - Samples drawn from apparently healthy volunteers were evaluated in this assay. No medical histories were available for the donors used in this study.

Note: *Samples were diluted prior to assay as described in the Sample Preparation section.*

CD40 Ligand

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	2650	100	583 - 4518
EDTA Plasma, platelet-poor (n=6)	ND	0	ND
Heparin Plasma, platelet-poor (n=6)	ND	0	ND

IFN-γ

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	ND	0	ND
EDTA Plasma (n=12)	ND	0	ND
Heparin Plasma (n=12)	ND	0	ND

IL-1α

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	ND	0	ND
EDTA Plasma (n=12)	ND	0	ND
Heparin Plasma (n=12)	ND	0	ND

IL-1β

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	ND	0	ND
EDTA Plasma (n=12)	ND	0	ND
Heparin Plasma (n=12)	ND	0	ND

ND = Non-detectable

IL-6

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	3.1	42	ND - 4.3
EDTA Plasma (n=12)	2.9	33	ND - 3.3
Heparin Plasma (n=12)	3.0	33	ND - 3.3

IL-8

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	13.6	100	5.6 - 23.9
EDTA Plasma (n=12)	4.6	50	ND - 5.2
Heparin Plasma (n=12)	6.4	75	ND - 9.3

IL-17

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	ND	0	ND
EDTA Plasma (n=12)	ND	0	ND
Heparin Plasma (n=12)	ND	0	ND

TNF-α

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	ND	0	ND
EDTA Plasma (n=12)	ND	0	ND
Heparin Plasma (n=12)	ND	0	ND

ND = Non-detectable

Cell Culture Supernates - HT1080 human fibrosarcoma cells were cultured in DMEM supplemented with 10% fetal bovine serum. The cells were cultured unstimulated or stimulated with 10 ng/mL of PMA. Aliquots were removed on day one and assayed in the Mosaic ELISA.

	CD40L (pg/mL)	IFN-γ (pg/mL)	IL-1α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-17 (pg/mL)	TNF-α (pg/mL)
Unstimulated	ND	ND	ND	9	672	2657	ND	ND
Stimulated	ND	14	86	197	3315	2805	ND	83

ND = Non-detectable

SPECIFICITY

This assay recognizes both natural and recombinant proteins. The following factors were assayed for cross-reactivity and interference in the Mosaic Human Cytokine Panel 1. Less than 1% cross-reactivity or interference was observed except where noted on the next page.

Recombinant human:
4-1BB
6Ckine
APRIL
BLC/BCA-1
BAFF/BLyS
CCL28
CD27 Ligand
CD30 Ligand
CKβ8-1
CLC
CTACK
CX_3CR_1
CXCL16
CXC-X3
EDA
EDA-A2
ENA-78
Eotaxin
Eotaxin-2
Eotaxin-3
Eotaxin-3 (aa 24-94)
Fas Ligand
Fractalkine
GCP-2
GITR Ligand
GROα
GROβ
GROγ
HCC-1
HCC-4
I-309
IL-1F5
IL-1F7
IL-1F9
IL-1HY2
IL-17B
IL-17C
IL-17D
IL-17E
IL-17F
IL-18
IP-10
I-TAC
LIGHT
LT-α1/β2
LT-α2/β1
Lymphotactin
MCP-1
MCP-2
MCP-3
MCP-4
MDC
MIG
MIG-2
MIG-6
MIP-1α
MIP-1α (70 aa)
MIP-1β
MIP-1δ (68 aa)
MIP-1δ (92 aa)
MIP-3α
MIP-3β
MPIF-1
NAP-2
OX40 Ligand
PARC
PF4
RANTES
SDF-1α
SDF-1β
TECK
TNF-β
TRAIL
TRANCE
TWEAK
VEGI
XCR-1

Recombinant mouse:
6Ckine
BAFF/BLyS
BLC/BCA-1
CCL6
CCL28
CD27 Ligand
CD30 Ligand
CD40 Ligand
CRG-2/IP-10
CTACK
CXCL3
CXCL16
CXC-X3
Eotaxin
Eotaxin-3like
Fas Ligand
Fractalkine
GCP-2
IL-1F6
IL-1F8
IL-18
I-TAC
JE/MCP-1
KC
LT-α1/β2
LT-α2/β1
Lungkine
Lymphotactin
MCP-3
MCP-5
MDC
MIG
MIP-1α
MIP-1β
MIP-1γ
MIP-2
MIP-3α
MIP-3β
OPG
OX40 Ligand
PF4
RANTES
SCF
SDF-1α
TARC
TCK-1
TECK
TNF-α
TNF-α (truncated)
TPO
TRAIL
TRANCE

Recombinant rat:
CINC-1
CINC-2α
CINC-3
Fractalkine
Leptin
LIX
MIP-3α
TCK-1
TNF-α

Recombinant cotton rat:
GRO
IFN-γ
IL-1α
IL-6
IP-10
MIP-1α
MIP-1β
RANTES
TNF-α

Recombinant bovine:
IFN-γ
TNF-α

Recombinant porcine:
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
TNF-α

Recombinant equine:
IFN-γ
IL-1β
IL-6
TNF-α

Recombinant canine:
IFN-γ
IL-1β
IL-6
IL-8
TNF-α

Recombinant feline:
IFN-γ
IL-6
IL-8
RANTES
TNF-α

Recombinant guinea pig:
TNF-α

Recombinant rhesus macaque:
TNF-α

Recombinant viral:
CMVUL147
MCV (II)
MIP-1
MIP-2
MIP-3

Cross-reactivity

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and displayed ≥ 1% cross-reactivity with the Mosaic Human Cytokine Panel 1.

Recombinant Feline IL-1β	1.0%
Recombinant Rhesus Macaque IL-1β	2.8%
Recombinant Rhesus Macaque IFN-γ	1.9%

Interference

The factors listed below were prepared at 50 ng/mL in a mid-range Mosaic Human Cytokine Panel 1 Standard and interfered with the Mosaic Human Cytokine Panel 1 at the levels indicated.

Recombinant Cotton Rat IL-1β	≤ 1 ng/mL
Recombinant Equine IL-8	> 1 ng/mL
Recombinant Porcine IL-8	≤ 1 ng/mL

Substrates 1 and 2 are comprised of TMA-6, a product of Lumigen, Inc., Southfield, Michigan, USA, and are covered by the following:

US Patent Numbers: 5,922,558 and 6,858,733

International Patent Numbers: 733,086, 1,019,525, 2,300,071, 1,015,461, 2,002,352,881, ZL02805225.0, and 1,456,716