Mosaic™ ELISA

Human Th1/Th2/Th17 Panel

Catalog Number MEA002

For the simultaneous quantitative determination of concentrations of multiple human T helper cell lineage markers 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.

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INTRODUCTION

$CD4^+$ T helper (Th) cells are critical mediators of the adaptive immune response. Naïve $CD4^+$ T cells are activated by antigen-presenting cells and subsequently differentiate into Th cell lineages. These include T helper type 1 (Th1) cells, Th2 cells, Th9 cells, Th17 cells, and Th22 cells. The various Th cell subsets are noted for their secretion of specific cytokines that define the function of the cell. Thus, the ability to analyze multiple cytokines simultaneously can be used for the rapid detection of different Th responses. The Mosaic Human Th1/Th2/Th17 ELISA is a powerful tool capable of simultaneously detecting 11different cytokines in the same sample. These include the Th1-related cytokines IL-2, IL-12 p70, IFN-γ, and TNF-α, the Th2-related cytokines IL-4, IL-5, and IL-10, and the Th17-related cytokines IL-7, IL-15, IL-17, and IL-22. Mosaic ELISA Kits employ a multiplex array in each well of a 96-well plate to provide an accurate, efficient, and economical alternative to conducting multiple traditional ELISA experiments.

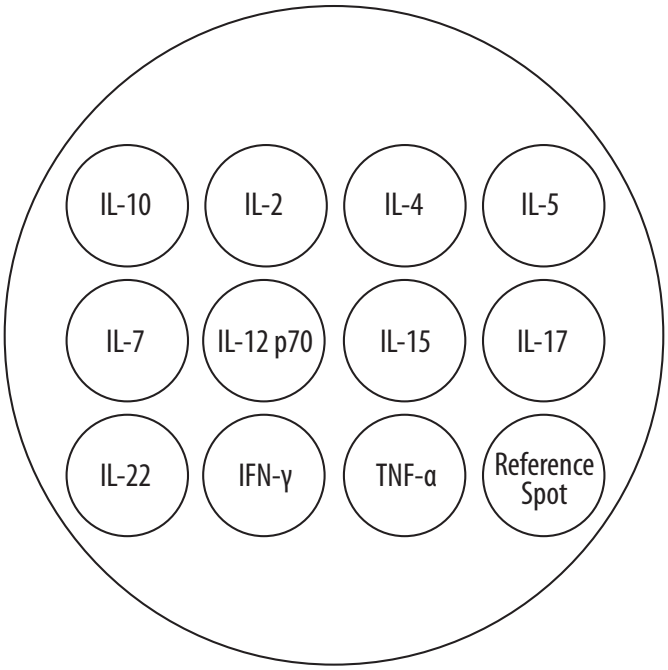


Figure 1: A visualization of the spot layout per well.

PRINCIPLE OF ASSAY

The Mosaic Human Th1/Th2/Th17 Panel Immunoassay employs a two-site sandwich ELISA technique to simultaneously detect eleven Th lineage markers in cell culture supernates, serum, and plasma. Multiple capture antibodies that specifically recognize the target Th lineage markers have been pre-spotted into each well of a 96 well microplate. Standards and samples are added, and Th lineage markers present in the samples are bound by the immobilized antibodies. After washing away unbound material, biotinylated detection antibodies are used to detect the specific Th lineage markers. 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- 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 eleven Th lineage markers in a complex sample. Multipurpose diluents are used to optimize recovery, linearity, and reproducibility. These diluents 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 ligands, proteins, and other factors present in biological samples. Until all factors have been tested, the possibility of interference cannot be excluded.
- Discrepancies may exist in values obtained for the same analyte utilizing different technologies.
- **Only the analytes listed on the enclosed Standard Value Card can be measured with this kit.**

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Th1/Th2/Th17 Microplate	894099	96 well microplate spotted with 11 antibodies against specific cytokines.	Invert the plate, and blot it against clean paper towels to dry the plate. Mark the used wells. Return the plate to the foil pouch containing the desiccant pack, and reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Standard	894101	2 vials of a cocktail of recombinant human cytokines in a buffered protein base with preservatives; lyophilized.	Discard after use. Use a fresh standard for each assay.
Detection Mix	894102	6 mL of a cocktail of antibodies conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-102	895939	6 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5K	895119	21 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-40	895817	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservatives.	
Streptavidin-HRP	895469	6 mL of a streptavidin-horseradish peroxidase conjugate with preservatives.	
Substrate 1	895471	3 mL of a buffered solution.	
Substrate 2	895472	3 mL of a buffered solution.	
Plate Sealers	N/A	8 adhesive strips.	Store at room temperature.
Standard Value Card	749224	1 card listing the standard reconstitution volume and concentrations for this lot of standard.	

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

OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips.
- Deionized or distilled water.
- 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 of standards and samples.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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.*
Heparin plasma is not recommended for use with IL-7.

SAMPLE PREPARATION

Serum and plasma samples require at least a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-40. Mix thoroughly.

REAGENT PREPARATION

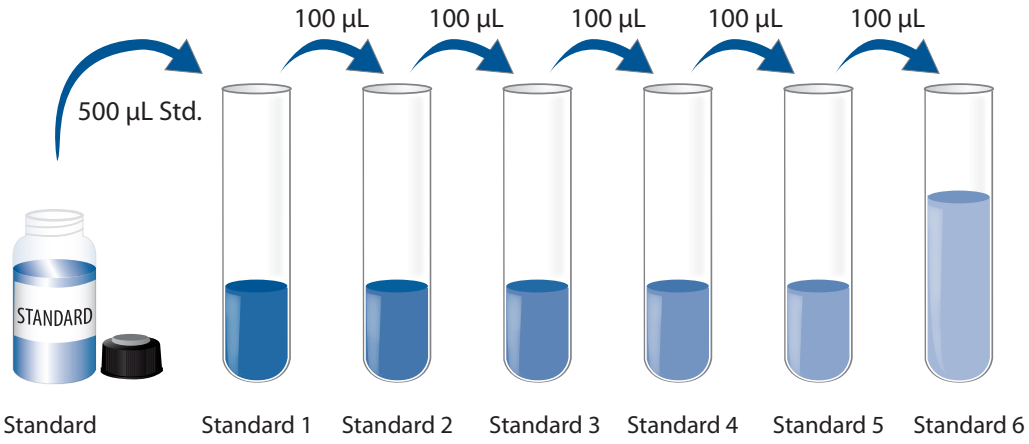
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 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 (f*or serum/plasma samples*). Refer to the Standard Value Card for the reconstitution volume and assigned values. 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 Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6-40 (f*or serum/plasma samples*) 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. A plate layout is provided as a record of standards and samples assayed.
   For Cell Culture Supernate samples: Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
   For Serum/Plasma samples: Incubate for 3 hours at room temperature on the shaker 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, manifold dispenser, or autowasher. Removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by decanting with force over a sink several times. Do not blot against paper towels as this may cause over drying of the wells.
5. Add 50 µL of the Detection Mix to all wells. Securely cover with a plate sealer and incubate for 1 hour 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 15 minutes at room temperature on the shaker set at 500 ± 50 rpm. **Protect from light.**
8. Repeat the wash as in step 4.
9. Add 50 µL of Substrate Solution to each well. **Protect from light.**
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.

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

Thirty 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)
IL-2	0.63	0.03-1.49
IL-4	0.67	0.28-1.56
IL-5	0.07	0.03-0.16
IL-7	0.12	0.05-0.23
IL-10	1.13	0.31-3.26
IL-12 p70	1.21	0.64-2.57
IL-15	0.18	0.09-0.54
IL-17	0.66	0.20-1.31
IL-22	0.55	0.19-1.02
IFN-γ	0.41	0.14-1.02
TNF-α	0.56	0.25-1.33

CALIBRATION

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

This assay has been correlated to the respective Quantikine® ELISA kit with slopes of 0.9-1.1 and R^2 values greater than 0.9.

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).

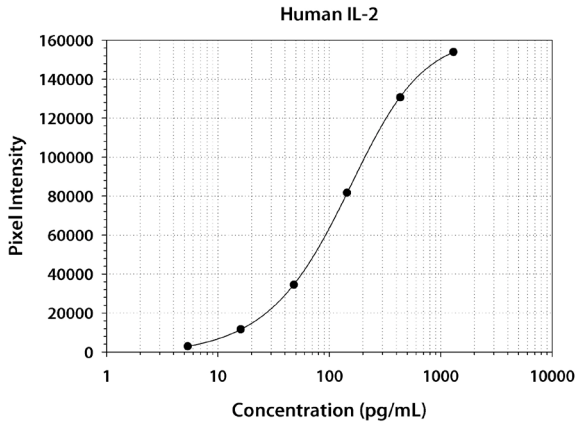
Create a standard curve for each analyte by reducing the data using computer software capable of generating a 5-PL curve fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

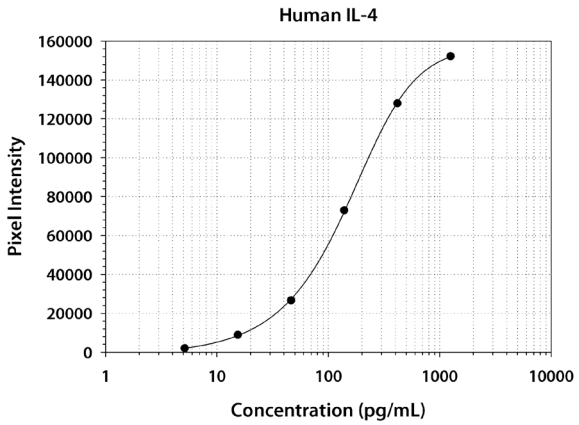
TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

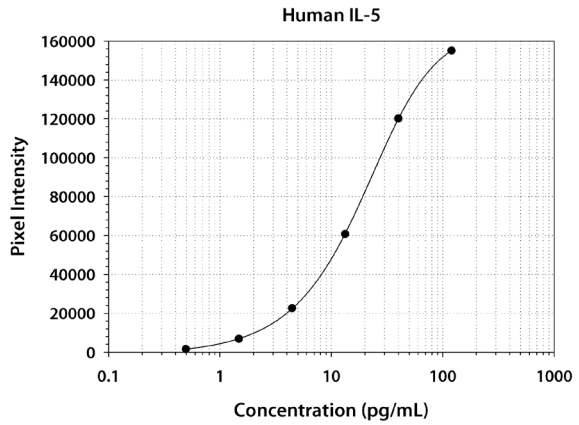
CALIBRATOR DILUENT RD6-40



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	2038 1818	1928	—
Standard 1	1300	156,070 155,633	155,852	153,924
Standard 2	433	132,424 132,726	132,575	130,647
Standard 3	144	84,472 82,787	83,630	81,702
Standard 4	48.1	36,828 35,991	36,410	34,482
Standard 5	16.0	13,871 13,216	13,544	11,616
Standard 6	5.35	4707 4912	4810	2882



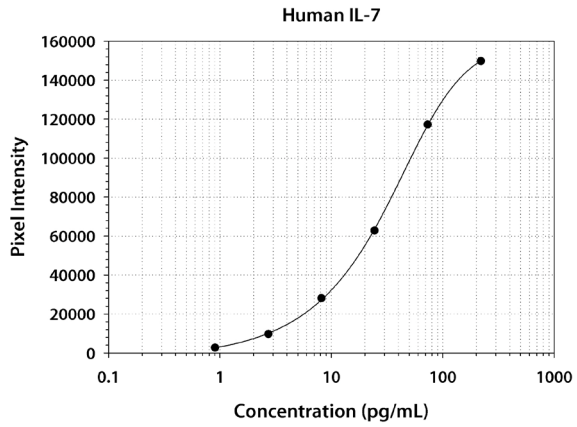
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1298 1394	1346	—
Standard 1	1250	152,853 154,234	153,544	152,198
Standard 2	417	129,873 128,748	129,311	127,965
Standard 3	139	74,732 73,831	74,282	72,936
Standard 4	46.3	28,132 27,771	27,952	26,606
Standard 5	15.4	10,389 10,079	10,234	8888
Standard 6	5.14	3216 3420	3318	1972



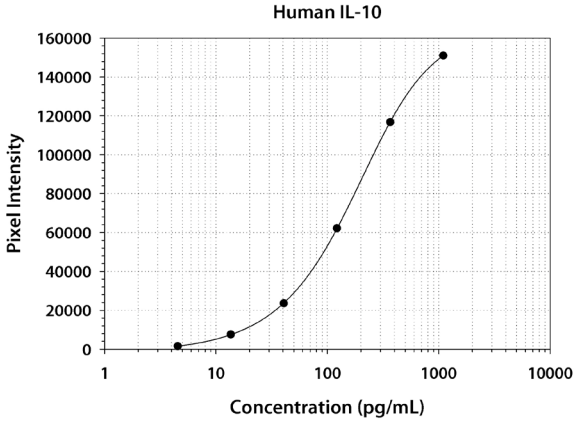
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1609 1557	1583	—
Standard 1	120	156,553 156,796	156,675	155,092
Standard 2	40.0	123,209 120,259	121,734	120,151
Standard 3	13.3	63,244 61,371	62,308	60,725
Standard 4	4.44	24,234 24,098	24,166	22,583
Standard 5	1.48	8438 8408	8423	6840
Standard 6	0.494	3150 3195	3173	1590

TYPICAL DATA *CONTINUED*

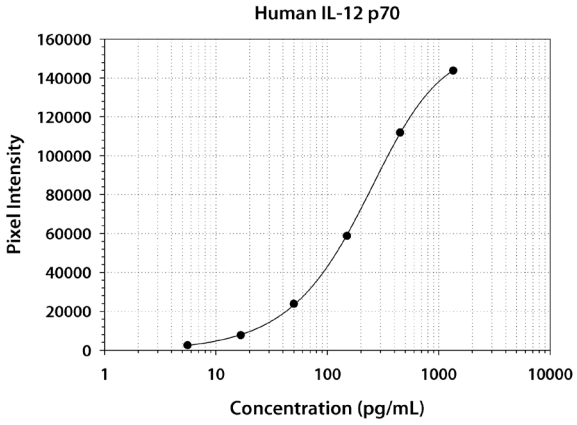
CALIBRATOR DILUENT RD6-40



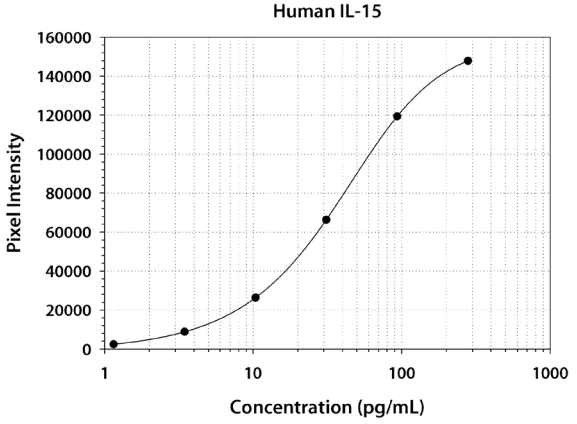
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1519 1671	1595	—
Standard 1	220	152,304 150,516	151,410	149,815
Standard 2	73.3	118,655 118,973	118,814	117,219
Standard 3	24.4	64,111 64,773	64,442	62,847
Standard 4	8.15	29,779 29,589	29,684	28,089
Standard 5	2.72	11,395 11,266	11,331	9736
Standard 6	0.905	4217 4376	4297	2702



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1418 1348	1383	—
Standard 1	1100	151,643 153,087	152,365	150,982
Standard 2	367	117,556 118,809	118,183	116,800
Standard 3	122	64,018 63,116	63,567	62,184
Standard 4	40.7	24,823 25,067	24,945	23,562
Standard 5	13.6	8963 8892	8928	7545
Standard 6	4.53	2843 2976	2910	1527



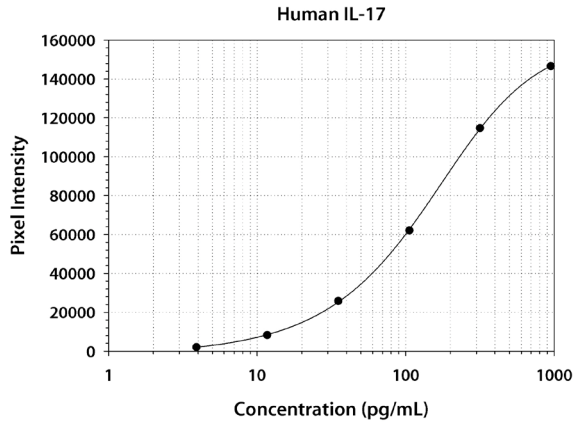
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	2287 2106	2197	—
Standard 1	1350	146,093 145,933	146,013	143,817
Standard 2	450	113,983 114,179	114,081	111,885
Standard 3	150	60,936 60,979	60,958	58,761
Standard 4	50	26,240 25,704	25,972	23,776
Standard 5	16.7	10,019 9797	9908	7712
Standard 6	5.56	4653 4732	4693	2496



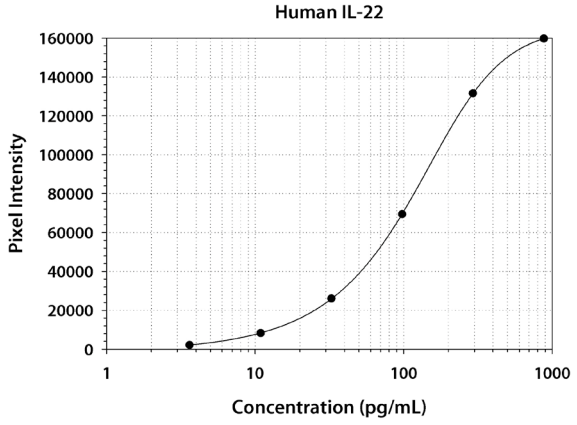
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1657 1556	1607	—
Standard 1	280	148,404 150,512	149,458	147,852
Standard 2	93.3	120,874 120,999	120,937	119,330
Standard 3	31.1	67,981 67,847	67,914	66,308
Standard 4	10.4	28,106 27,789	27,948	26,341
Standard 5	3.46	10,556 10,326	10,441	8835
Standard 6	1.15	4005 4049	4027	2421

TYPICAL DATA *CONTINUED*

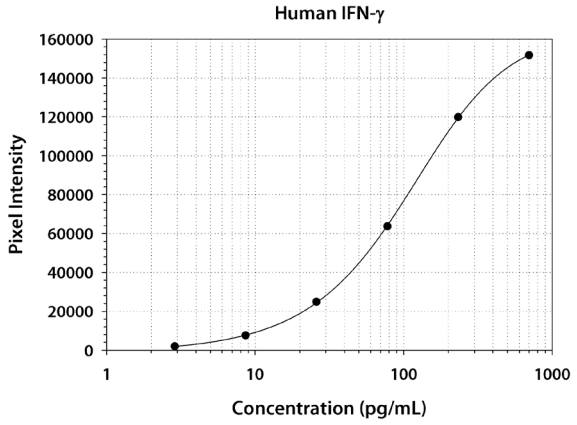
CALIBRATOR DILUENT RD6-40



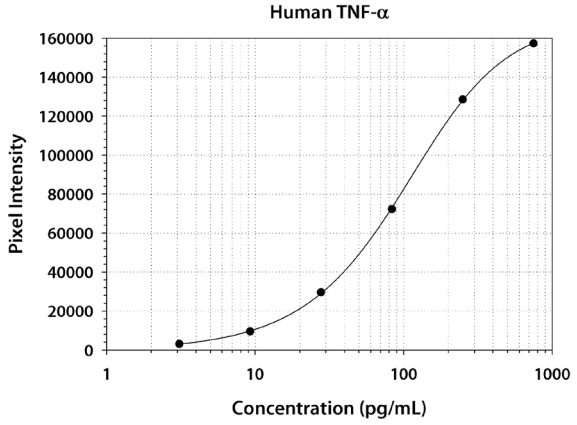
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1291 1399	1345	—
Standard 1	950	148,256 147,573	147,915	146,570
Standard 2	317	115,490 116,634	116,062	114,717
Standard 3	106	63,114 63,795	63,455	62,110
Standard 4	35.2	27,787 26,454	27,121	25,776
Standard 5	11.7	9464 9685	9575	8230
Standard 6	3.91	3319 3477	3398	2053



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1417 1390	1404	—
Standard 1	880	161,177 161,170	161,174	159,770
Standard 2	293	133,613 132,334	132,974	131,570
Standard 3	97.8	70,518 71,094	70,806	69,403
Standard 4	32.6	27,666 27,216	27,441	26,038
Standard 5	10.9	9097 10,207	9652	8249
Standard 6	3.62	3625 3477	3551	2148



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1487 1292	1390	—
Standard 1	700	152,956 153,268	153,112	151,723
Standard 2	233	122,149 120,300	121,225	119,835
Standard 3	77.8	65,107 64,962	65,035	63,645
Standard 4	25.9	26,080 26,386	26,233	24,844
Standard 5	8.64	9024 8843	8934	7544
Standard 6	2.88	3355 3312	3334	1944



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1460 1358	1409	—
Standard 1	750	158,976 158,490	158,733	157,324
Standard 2	250	130,007 129,904	129,956	128,547
Standard 3	83.3	74,353 73,101	73,727	72,318
Standard 4	27.8	30,836 31,168	31,002	29,593
Standard 5	9.26	11,177 10,685	10,931	9522
Standard 6	3.09	4666 4348	4507	3098

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 fifty-three separate assays to assess inter-assay precision.

IL-2	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	23.2	101	398	23.5	95.4	366
Standard deviation	1.20	5.63	20.0	3.3	12.2	39.0
CV (%)	5.2	5.6	5.0	13.9	12.8	10.7

IL-4	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	14.7	49.3	235	15.3	49.5	228
Standard deviation	0.68	1.84	11.6	1.79	5.32	20.5
CV (%)	4.6	3.7	4.9	11.7	10.7	9.0

IL-5	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	2.72	10.4	37.5	2.86	11.6	40.4
Standard deviation	0.22	1.01	3.50	0.36	1.44	4.00
CV (%)	8.0	9.7	9.3	12.4	12.4	9.9

IL-7	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	2.33	10.9	48.6	2.55	11.9	48.7
Standard deviation	0.19	0.68	3.41	0.35	1.33	4.08
CV (%)	8.2	6.2	7.0	13.7	11.2	8.4

IL-10	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	15.2	54.8	230	14.3	58.2	243
Standard deviation	1.33	2.40	15.6	1.80	6.02	23.9
CV (%)	8.7	4.4	6.8	12.6	10.3	9.8

PRECISION *CONTINUED*

IL-12 p70	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	31.4	100	446	30.1	97.1	417
Standard deviation	2.18	6.59	34.6	3.61	9.90	42.1
CV (%)	6.9	6.6	7.8	12.0	10.2	10.1

IL-15	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	6.67	25.8	97.9	6.75	26.1	93.2
Standard deviation	0.34	1.57	5.12	0.72	2.52	8.90
CV (%)	5.1	6.1	5.2	10.6	9.6	9.5

IL-17	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	6.66	26.4	77.6	5.38	23.9	72.5
Standard deviation	0.66	1.91	4.93	0.80	3.01	7.78
CV (%)	9.8	7.2	6.3	14.9	12.6	10.7

IL-22	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	29.9	128	436	31.0	130	407
Standard deviation	0.91	6.23	30.0	2.98	12.1	60.2
CV (%)	3.1	4.9	6.9	9.6	9.3	14.8

IFN-γ	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	8.71	35.3	140	9.35	38.9	140
Standard deviation	0.50	1.80	9.42	1.37	4.93	15.9
CV (%)	5.7	5.1	6.7	14.7	12.7	11.4

TNF-α	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	53	53	53
Mean (pg/mL)	5.68	30.1	132	6.40	34.3	137
Standard deviation	0.40	1.92	9.67	1.05	5.10	17.3
CV (%)	7.1	6.4	7.3	16.4	14.8	12.7

RECOVERY

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

IL-2

Sample Type	Average % Recovery	Range
Cell culture supernates	93	86-103%
Serum*	99	84-106%
EDTA plasma*	93	81-101%
Heparin plasma*	97	78-109%

IL-4

Sample Type	Average % Recovery	Range
Cell culture supernates	96	90-107%
Serum*	96	85-102%
EDTA plasma*	93	87-101%
Heparin plasma*	89	77-101%

IL-5

Sample Type	Average % Recovery	Range
Cell culture supernates	100	94-108%
Serum*	102	92-109%
EDTA plasma*	95	88-99%
Heparin plasma*	86	81-97%

IL-7

Sample Type	Average % Recovery	Range
Cell culture supernates	94	81-103%
Serum*	113	105-126%
EDTA plasma*	109	104-114%

IL-10

Sample Type	Average % Recovery	Range
Cell culture supernates	102	99-108%
Serum*	112	100-118%
EDTA plasma*	111	100-117%
Heparin plasma*	110	86-126%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

RECOVERY *CONTINUED*

IL-12 p70

Sample Type	Average % Recovery	Range
Cell culture supernates	98	93-105%
Serum*	108	106-116%
EDTA plasma*	101	95-110%
Heparin plasma*	98	94-107%

IL-15

Sample Type	Average % Recovery	Range
Cell culture supernates	78	70-91%
Serum*	96	93-102%
EDTA plasma*	99	91-107%
Heparin plasma*	97	88-112%

IL-17

Sample Type	Average % Recovery	Range
Cell culture supernates	87	79-98%
Serum*	122	115-127%
EDTA plasma*	108	104-112%
Heparin plasma*	111	102-125%

IL-22

Sample Type	Average % Recovery	Range
Cell culture supernates	102	94-109%
Serum*	82	70-92%
EDTA plasma*	83	78-88%
Heparin plasma*	81	74-87%

IFN-γ

Sample Type	Average % Recovery	Range
Cell culture supernates	119	108-129%
Serum*	100	95-104%
EDTA plasma*	101	96-106%
Heparin plasma*	80	76-84%

TNF-α

Sample Type	Average % Recovery	Range
Cell culture supernates	98	96-102%
Serum*	98	85-105%
EDTA plasma*	98	92-111%
Heparin plasma*	98	80-108%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

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

IL-2

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	112	119	124	105
	Range (%)	104-123	115-125	105-136	99-110
1:4	Average % of Expected	108	107	112	102
	Range (%)	104-114	104-109	104-119	91-114
1:8	Average % of Expected	108	107	113	103
	Range (%)	101-118	103-112	102-123	87-121

IL-4

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	112	113	118	100
	Range (%)	107-116	102-121	103-127	93-105
1:4	Average % of Expected	116	114	113	105
	Range (%)	113-120	109-118	96-127	96-115
1:8	Average % of Expected	121	115	116	110
	Range (%)	119-125	111-122	101-130	99-123

IL-5

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	109	99	111	99
	Range (%)	106-112	95-104	105-125	78-110
1:4	Average % of Expected	109	103	109	108
	Range (%)	102-113	100-109	92-127	98-119
1:8	Average % of Expected	113	102	108	115
	Range (%)	100-120	94-108	98-134	97-140

IL-7

		Cell culture supernates	Serum*	EDTA plasma*
1:2	Average % of Expected	111	99	108
	Range (%)	101-119	93-105	106-114
1:4	Average % of Expected	125	102	110
	Range (%)	124-126	97-106	101-121
1:8	Average % of Expected	131	99	109
	Range (%)	128-133	96-103	99-126

*Samples were diluted prior to assay.

LINEARITY *CONTINUED*

IL-10

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	102	99	104	109
	Range (%)	96-106	80-108	96-121	98-123
1:4	Average % of Expected	96	100	102	108
	Range (%)	91-100	83-118	90-117	99-122
1:8	Average % of Expected	96	95	104	104
	Range (%)	88-102	78-108	92-123	98-114

IL-12 p70

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	110	103	102	113
	Range (%)	104-116	100-106	98-106	97-133
1:4	Average % of Expected	106	94	100	114
	Range (%)	104-108	88-97	95-109	94-126
1:8	Average % of Expected	107	101	100	119
	Range (%)	103-113	94-116	96-105	108-132

IL-15

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	114	114	107	102
	Range (%)	110-120	108-121	100-113	95-105
1:4	Average % of Expected	119	115	103	101
	Range (%)	116-124	110-120	99-108	95-104
1:8	Average % of Expected	127	110	106	105
	Range (%)	123-131	105-117	101-113	100-109

IL-17

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	112	102	121	112
	Range (%)	107-116	89-112	114-128	93-126
1:4	Average % of Expected	115	98	108	110
	Range (%)	104-131	84-106	97-118	97-125
1:8	Average % of Expected	112	94	100	100
	Range (%)	105-120	86-104	87-114	84-113

*Samples were diluted prior to assay.

LINEARITY *CONTINUED*

IL-22

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	111	111	106	107
	Range (%)	107-114	107-116	102-112	103-110
1:4	Average % of Expected	105	111	107	105
	Range (%)	104-107	103-122	104-111	97-111
1:8	Average % of Expected	102	109	104	111
	Range (%)	98-107	99-117	98-110	109-113

IFN-γ

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	109	107	112	111
	Range (%)	104-117	103-110	101-133	105-117
1:4	Average % of Expected	102	105	110	115
	Range (%)	96-112	101-111	100-127	100-127
1:8	Average % of Expected	103	103	102	120
	Range (%)	96-118	97-110	87-123	112-131

TNF-α

		Cell culture supernates	Serum*	EDTA plasma*	Heparin plasma*
1:2	Average % of Expected	108	108	107	107
	Range (%)	104-114	103-110	99-114	96-115
1:4	Average % of Expected	103	108	107	108
	Range (%)	99-109	104-115	99-113	97-120
1:8	Average % of Expected	108	110	104	111
	Range (%)	101-112	105-118	101-108	98-120

*Samples were diluted prior to assay.

SAMPLE VALUES

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

IL-7

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum* (n=20)	8.40	100	2.80-18.3
EDTA plasma* (n=20)	1.74	10	ND-1.87
Heparin plasma* (n=20)	NR	NR	NR

IL-15

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum* (n=20)	———	0	———
EDTA plasma* (n=20)	———	0	———
Heparin plasma* (n=20)	2.75	5	———

TNF-α

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum* (n=20)	6.65	15	ND-6.90
EDTA plasma* (n=20)	———	0	———
Heparin plasma* (n=20)	6.49	10	ND-6.60

ND=Non-detectable

NR=Not Recommended

*Samples were diluted prior to assay as directed in the Sample Preparation section.

Twenty serum and plasma samples were tested and no detectable levels of IL-2, IL-4, IL-5, IL-10, IL-12 p70, IL-17, IL-22, and IFN-γ were observed.

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMC; 1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL PHA for 3 days. An aliquot of the cell culture supernate was removed and assayed in the Mosaic ELISA.

Cell Line	(pg/mL)										
	IL-2	IL-4	IL-5	IL-7	IL-10	IL-12 p70	IL-15	IL-17	IL-22	IFN-γ	TNF-α
PBMC + PHA	18.5	10.7	>110	ND	676	5.57	3.31	48.8	ND	>710	>770

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant proteins. This assay also recognizes recombinant canine IL-10.

The following factors were assayed for cross-reactivity and interference in the Mosaic Human Th1/Th2/Th17 Panel. Less than 1% cross-reactivity or interference was observed.

Recombinant human:

ANG
Ang-2
BDNF
β-ECGF
CD4
CD40 Ligand
CNTF
Common γ Chain
CT-1
CTLA-4
CXCL8/IL-8
EGF
Fas
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
G-CSF
G-CSF R
GDNF
GITR
GITR Ligand
GM-CSF
gp130
GROα
GROβ
HB-EGF
HGF
IGF-I
IGF-II
IL-1α
IL-1β
IL-1 ra
IL-1 RI
IL-1 RII
IL-2 Rα
IL-2 Rβ
IL-3
IL-3 Rα
IL-5 Rα
IL-5 Rβ
IL-6
IL-6 R
IL-7 R
IL-9
IL-10 Rα
IL-10 Rβ
IL-11
IL-11 Rα
IL-12 p40
IL-16
IL-17B
IL-17C
IL-17D
IL-17E
IL-17 R
IL-19
IL-20
IL-23
IL-24
IL-26
IL-28A/IFN-λ2
IL-29
KGF/FGF-7
LAP (TGF-β1)
Leptin
LIF
LIF Rα
MCP-1
M-CSF
MIF
MIP-1α
MIP-1β
NT-3
OPG
OSM
PD-ECDF
PD-ECGF
PDGF-AB
PDGF-BB
RANTES
SCF
SLPI
SMDF
TGF-α
TGF-β1
TGF-β2
TGF-β3
TGF-β RII
TNF-β
Tpo
TRAIL
TRANCE
$VEGF_{121}$
$VEGF_{165}$

Recombinant mouse:

CT-1
CTLA-4
Fas
Fas Ligand
GM-CSF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-9
IL-10
IL-11
IL-11 Rα
IL-12 p40
IL-13
IL-17
IL-23
Leptin
LIF
LIF Rα
MIP-1β
OPG
OPN
OSM
TNF-α
Tpo
TRANCE

Recombinant rat:

CNTF
IFN-γ
IL-1α
IL-4
IL-10
IL-22
Leptin
PDGF-AA
TNF-α

Recombinant porcine:

IL-1α
IL-2
IL-4
IL-5
IL-6
IL-8
IL-10
TNF-α

Recombinant feline:

IL-5
IL-10

Recombinant equine:

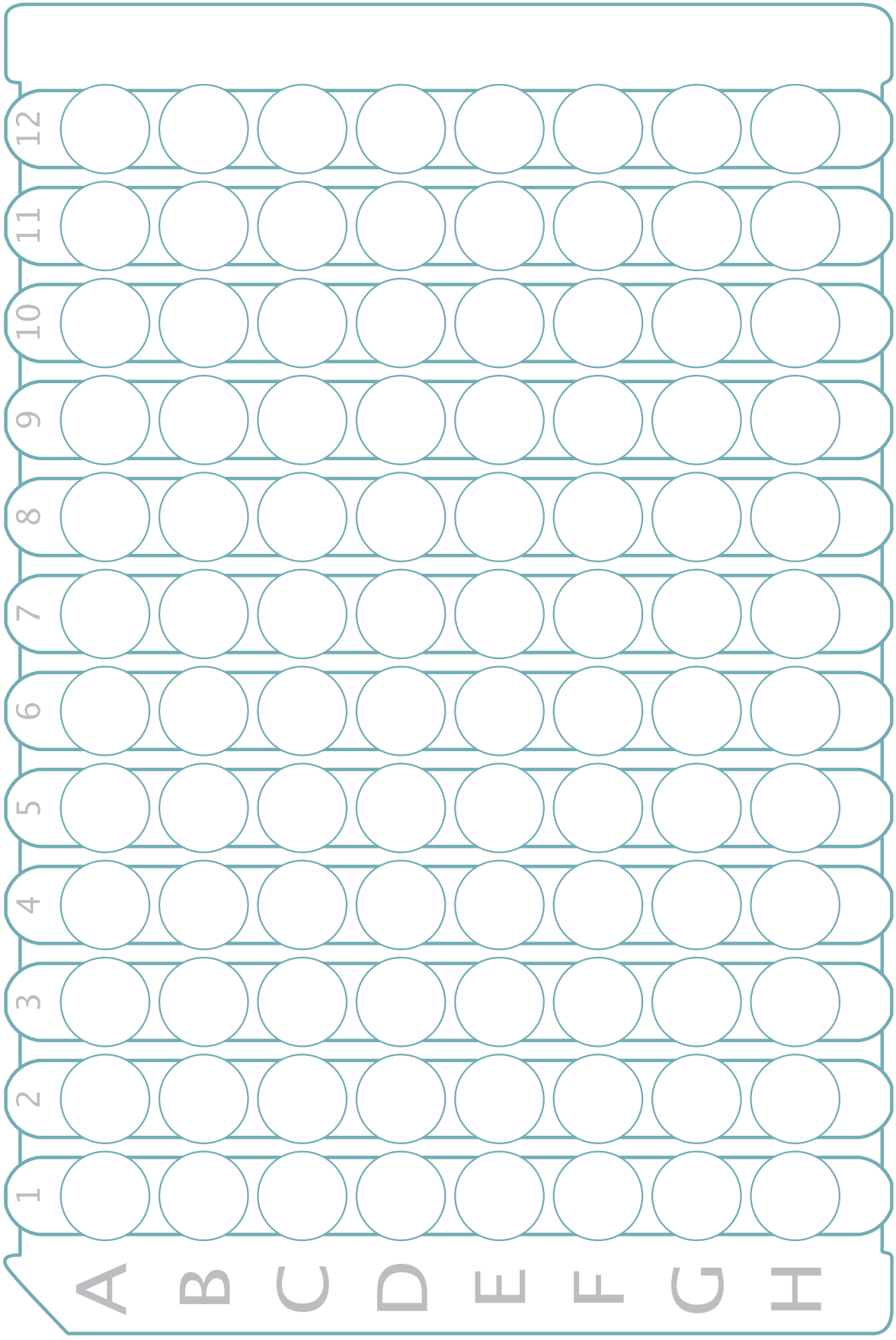
IL-5
IL-10

Other recombinants:

r. macaque IFN-γ
canine IL-5
viral IL-10
amphibian TGF-β5

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES

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