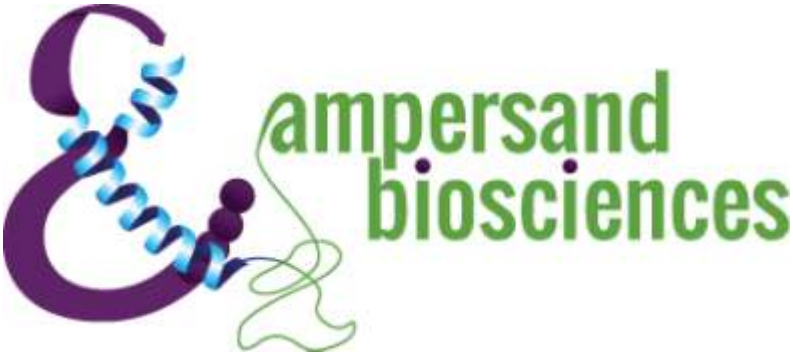
Human Cytokine Panel 3

Kit # HU120-K

Validation Report Version 1.0



Prepared by: Kristi Marshall Kristi Marshall (May 29, 2025 13:31 EDT) Date 29/05/25

Reviewed by: Laura Stephen Date 29/05/25

1. Assay Description:

A multiplex assay was developed and validated for the measurement of Human GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-22, IL-23, IL-27, IL-29, IL-31 and IL-9 The kit is microsphere-based and consist of using antigen-specific antibodies covalently coupled to magnetic Luminex beads and biotinylated detection antibodies in a capture-sandwich format. All incubations take place at room temperature in a 96-well plate. 30 µL of standard, controls or sample are added to the appropriate wells, followed by 10 µL of blocker and 10 µL of multiplexed capture-antibody microspheres. The plate is incubated for 1 hour at ambient temperature on a plate shaker. After washing 3 times, 40µL of detection antibodies are added to each well, thoroughly mixed, and incubated 1 hour at ambient temperature on a plate shaker. The Streptavidin-Phycoerythrin conjugate (SA-PE) working solution is then added to the plate and incubated for 30 minutes. The plate is then washed 3 times and the beads are resuspended in 100 µL of wash buffer. After shaking on a plate shaker for 10 minutes, the plate is then analyzed on the Luminex 200 Analyzer.

2. Control and Sample Description:

Control	Description
Control 1	Normal Human Serum (20%) spiked with low-levels of Recombinant GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-23, IL-27, IL-29, IL-31 and IL-9.
Control 2	Normal Human Serum (20%) spiked with mid-levels of Recombinant GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-22, IL-23, IL-27, IL-29, IL-31 and IL-9.

Sample	Description
Serum 1	Normal Human Serum spiked with Recombinant GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-23, IL-27, IL-29, IL-31 and IL-9.
Serum 2	Normal Human Serum spiked with Recombinant GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-23, IL-27, IL-29, IL-31 and IL-9.
Plasma 1	Normal Human Plasma spiked with Recombinant GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-23, IL-27, IL-29, IL-31 and IL-9.
Plasma 2	Normal Human Plasma spiked with Recombinant GM-CSF, IFNα, IFNβ, IL-12p70, IL-17, IL-21, IL-23, IL-27, IL-29, IL-31 and IL-9.

3. LLOQ, LDD and Curves:

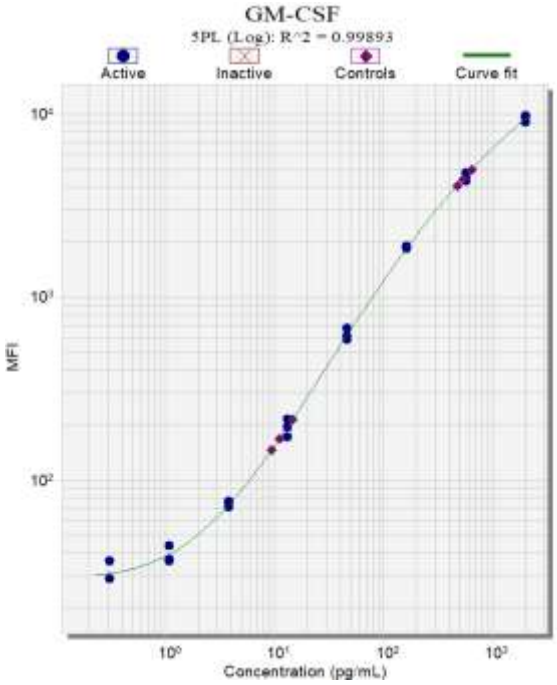
LDD: MFI (Median Fluorescent Intensity) for 8 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

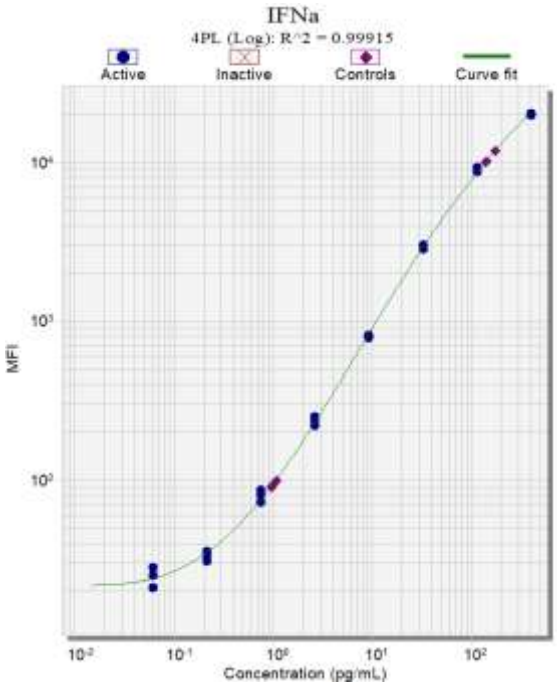
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in duplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

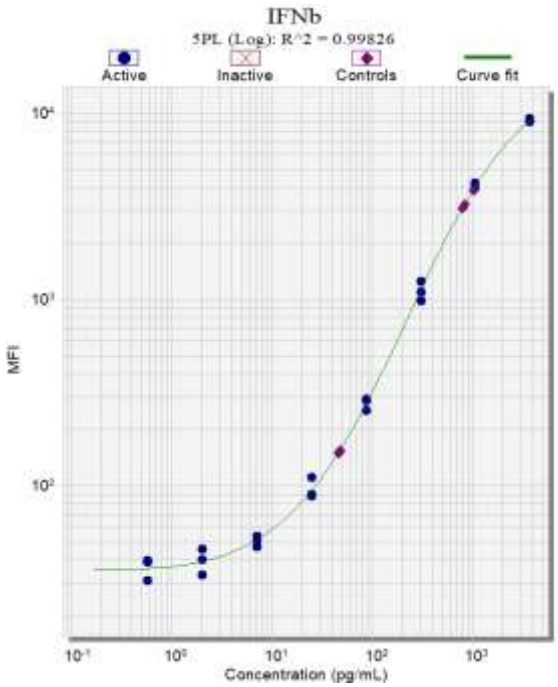
		S1	S8	LDD	LLOQ
GM-CSF	**pg/ml**	0.31	2000	0.73	1.6
IFNα	**pg/ml**	0.062	400	0.11	0.12
IFNβ	**pg/ml**	0.58	3750	7.0	13
IL-12p70	**pg/ml**	0.31	2000	1.3	2.0
IL-17	**pg/ml**	0.14	900	0.57	0.78
IL-21	**pg/ml**	0.23	1500	0.45	1.6
IL-22	**pg/ml**	0.31	2000	1.2	2.8
IL-23	**pg/ml**	1.3	8500	3.7	14
IL-27	**pg/ml**	1.2	7500	3.7	11
IL-29	**pg/ml**	0.039	250	0.23	0.36
IL-31	**pg/ml**	0.47	3000	1.8	2.4
IL-9	**pg/ml**	0.16	1000	0.83	1.9

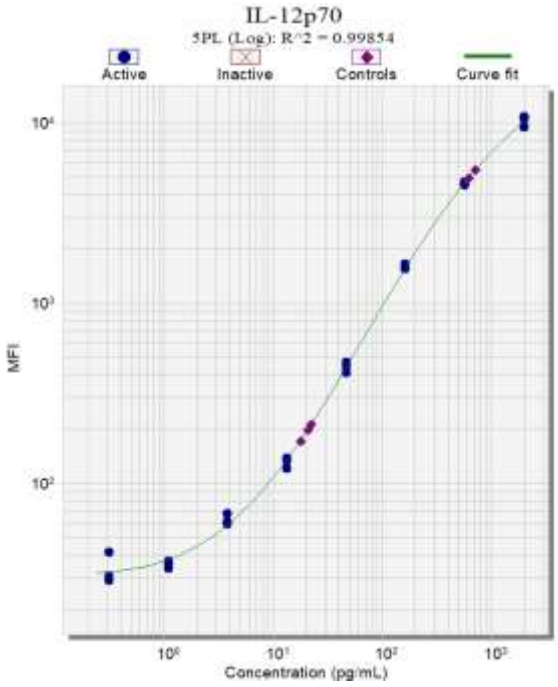
X Dilution Factor		S1	S8	LDD	LLOQ
GM-CSF	**pg/ml**	1.6	10000	3.6	8.1
IFNα	**pg/ml**	0.31	2000	0.53	0.59
IFNβ	**pg/ml**	2.9	18750	35	64
IL-12p70	**pg/ml**	1.6	10000	6.5	10
IL-17	**pg/ml**	0.70	4500	2.8	3.9
IL-21	**pg/ml**	1.2	7500	2.2	7.8
IL-22	**pg/ml**	1.6	10000	5.9	14
IL-23	**pg/ml**	6.6	42500	18	70
IL-27	**pg/ml**	5.8	37500	18	53
IL-29	**pg/ml**	0.19	1250	1.1	1.8
IL-31	**pg/ml**	2.3	15000	8.8	12
IL-9	**pg/ml**	0.78	5000	4.1	9.3

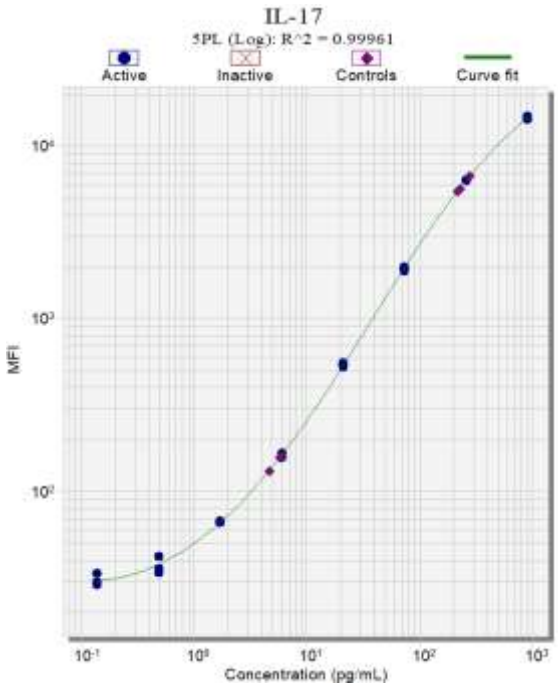
Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.

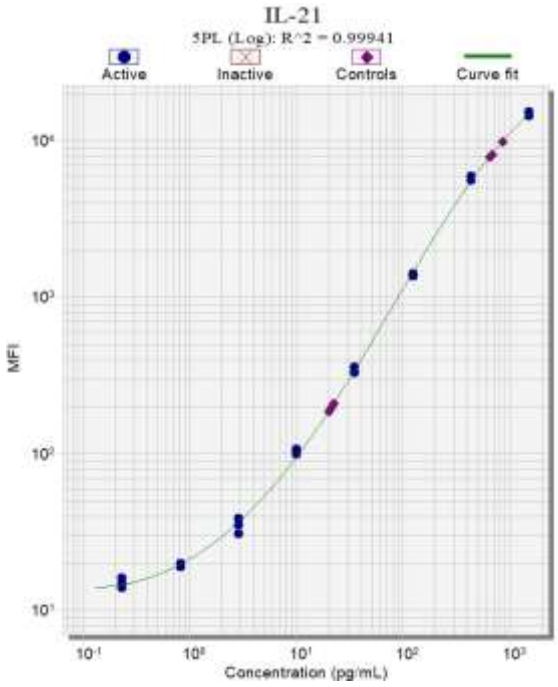


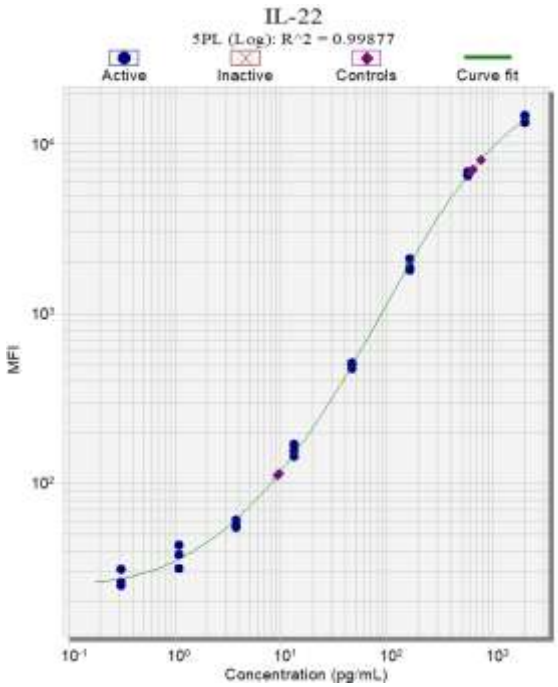


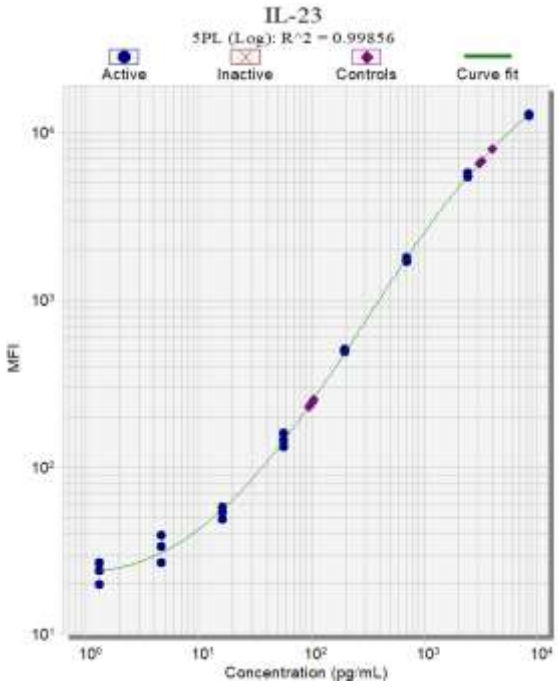

IFNb
5PL (Log): R^2 = 0.99826
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)

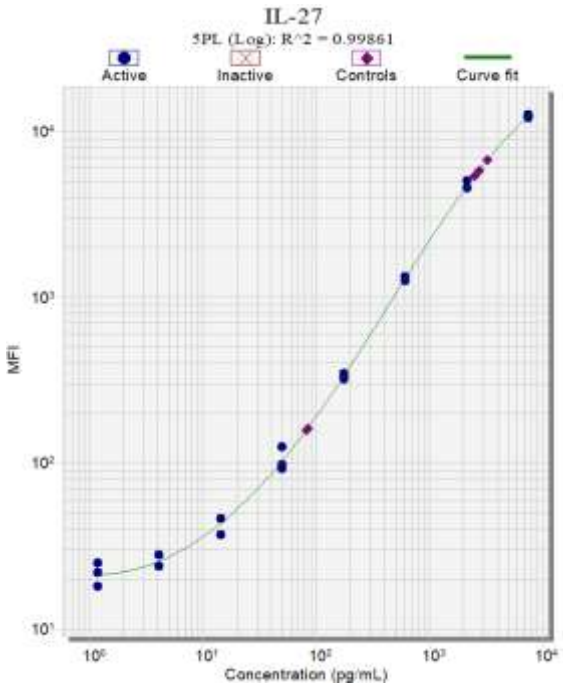

IL-12p70
5PL (Log): R^2 = 0.99854
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-17
5PL (Log): R^2 = 0.99961
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-21
5PL (Log): R^2 = 0.99941
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-22
5PL (Log): R^2 = 0.99877
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-23
5PL (Log): R^2 = 0.99856
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)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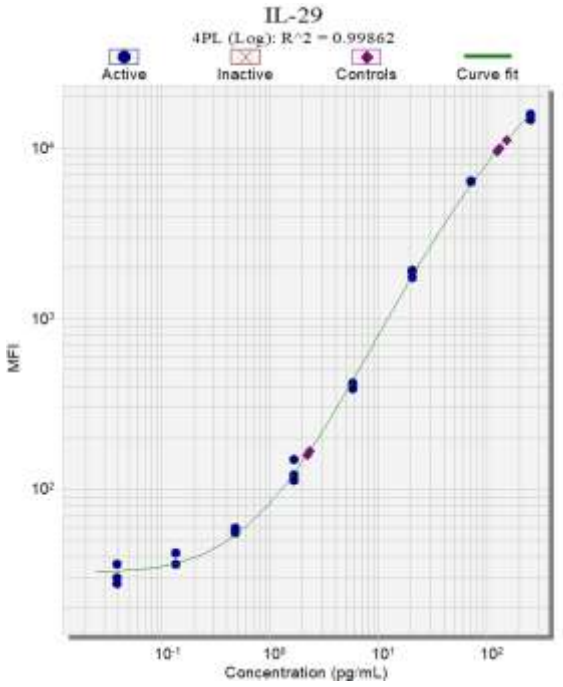
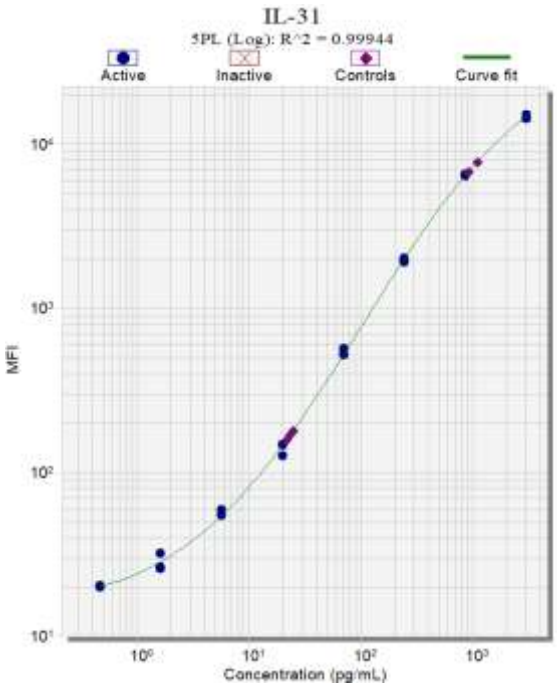
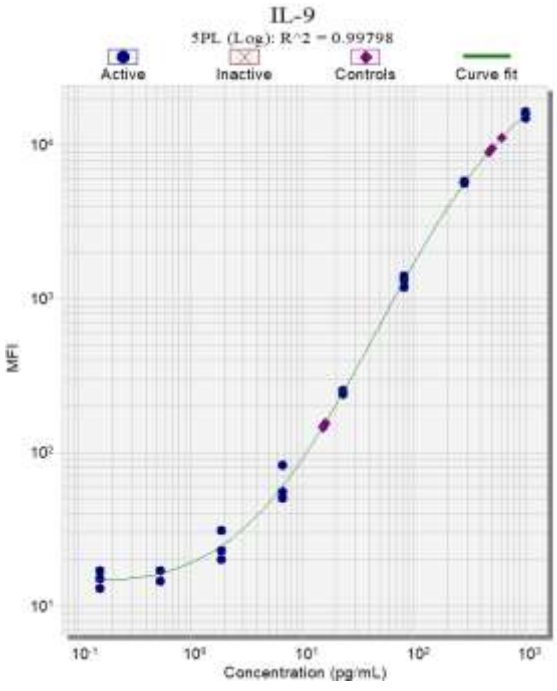
5PL (Log): R^2 = 0.99861
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-29
4PL (Log): R^2 = 0.99862
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-31
5PL (Log): R^2 = 0.99944
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)


IL-9
5PL (Log): R^2 = 0.99798
Active
Inactive
Controls
Curve fit
MFI
Concentration (pg/mL)

4. Precision:

Control samples were run in triplicate over 3 runs over 2 days with 2 analysts. Precision is the % CV of each run (intra, each run; inter, over 3 runs). Acceptance for precision is <20% CV. All assays meet acceptance for precision.

GM-CSF		1	2	3	Inter
Control 1	**Mean**	11	12	11	**12**
	% CV	4%	15%	17%	**12%**
Control 2	**Mean**	496	507	449	**484**
	% CV	17%	17%	12%	**9%**

IFNα		1	2	3	Inter
Control 1	**Mean**	1.0	1.0	1.0	**1.0**
	% CV	11%	4%	7%	**7%**
Control 2	**Mean**	129	129	140	**133**
	% CV	5%	5%	3%	**6%**

IFNβ		1	2	3	Inter
Control 1	**Mean**	48	53	52	**51**
	% CV	9%	13%	6%	**10%**
Control 2	**Mean**	790	844	897	**844**
	% CV	7%	10%	6%	**9%**

IL-12p70		1	2	3	Inter
Control 1	**Mean**	20	21	20	**20**
	% CV	8%	9%	5%	**7%**
Control 2	**Mean**	608	598	604	**603**
	% CV	18%	8%	5%	**10%**

IL-17		1	2	3	Inter
Control 1	**Mean**	5.9	6.5	6.0	**6.1**
	% CV	10%	10%	12%	**11%**
Control 2	**Mean**	220	229	235	**228**
	% CV	7%	6%	5%	**6%**

IL-21		1	2	3	Inter
Control 1	**Mean**	22	22	21	**22**
	% CV	6%	4%	8%	**5%**
Control 2	**Mean**	695	666	733	**698**
	% CV	9%	3%	4%	**7%**

IL-22		1	2	3	Inter
Control 1	**Mean**	10	10	9.0	**9.5**
	% CV	9%	10%	5%	**8%**
Control 2	**Mean**	623	597	647	**622**
	% CV	4%	1%	9%	**6%**

IL-23		1	2	3	Inter
Control 1	**Mean**	130	110	112	**117**
	% CV	7%	1%	7%	**10%**
Control 2	**Mean**	3427	3423	3293	**3381**
	% CV	10%	5%	6%	**7%**

IL-27		1	2	3	Inter
Control 1	**Mean**	93	82	88	**88**
	% CV	5%	10%	6%	**8%**
Control 2	**Mean**	2793	2793	2970	**2852**
	% CV	7%	7%	1%	**6%**

IL-29		1	2	3	Inter
Control 1	**Mean**	2.6	2.4	2.4	**2.4**
	% CV	7%	8%	4%	**7%**
Control 2	**Mean**	122	114	144	**127**
	% CV	5%	4%	8%	**12%**

IL-31		1	2	3	Inter
Control 1	**Mean**	26	26	26	**26**
	% CV	11%	4%	5%	**7%**
Control 2	**Mean**	889	885	970	**915**
	% CV	9%	5%	4%	**7%**

IL-9		1	2	3	Inter
Control 1	**Mean**	17	16	16	**16**
	% CV	9%	5%	5%	**7%**
Control 2	**Mean**	506	497	561	**521**
	% CV	8%	3%	5%	**8%**

5. Linearity:

Linearity was assessed using 2 serum, 2 plasma and 2 CSF samples spiked with the standard and diluted 1:2 for 4 dilutions. Percent Recovery was calculated using the calculated value (with kit dilution) as expected (observed x dilution / expected concentration X 100). The acceptance range for linearity is 70-130% recovery for all values above the LLOQ. All assays meet acceptance criteria.

GM-CSF				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	70	2385	1895	648
1:10	36	882	843	332
1:20	21	431	513	203
1:40	11	211	229	101
10	103%	74%	89%	102%
20	123%	72%	108%	125%
40	125%	71%	97%	125%

IFNα				
pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
1:5	9.4	732.0	7.8	203.5
1:10	4.7	305.5	3.6	98.0
1:20	2.6	196.0	2.1	52.3
1:40	1.4	114.5	0.74	25.9
10	100%	83%	93%	96%
20	109%	107%	108%	103%
40	115%	125%	76%	102%

IFNβ				
pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
1:5	3825	5865	12100	2505
1:10	2315	2690	5780	1195
1:20	1190	1043	3875	577
1:40	568	537	1890	372
10	121%	92%	96%	95%
20	124%	71%	128%	92%
40	119%	73%	125%	119%
IL-12p70				
pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
1:5	81	1565	829	1250
1:10	39	797	333	618
1:20	17	433	150	339
1:40	12	208	84	167
10	97%	102%	80%	99%
20	86%	111%	72%	108%
40	120%	106%	81%	107%

IL-17				
pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
1:5	861	848	42	303
1:10	521	333	19	165
1:20	257	164	12	87
1:40	127.5	93	6.0	45
10	121%	78%	93%	109%
20	119%	77%	114%	115%
40	118%	87%	114%	119%

IL-21				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	97	3055	64	849
1:10	49	1560	36	441
1:20	24	758	19	229
1:40	12	420	8.4	112
10	101%	102%	112%	104%
20	101%	99%	119%	108%
40	102%	110%	105%	106%

IL-22				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	536	2485	350	836
1:10	255	1415	177	452
1:20	110	702	109	221
1:40	66	393	55	119
10	95%	114%	101%	108%
20	82%	113%	124%	106%
40	98%	127%	126%	114%

IL-23				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	18450	238	1380	8505
1:10	8235	114	836	4585
1:20	3750	45	416	2525
1:40	1825	<LLOQ	169	948
10	89%	96%	121%	108%
20	81%	76%	121%	119%
40	79%	<LLOQ	98%	89%

IL-27				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	436	18900	2125	13750
1:10	217	7920	1215	7280
1:20	112	4500	618	3890
1:40	49	2140	307	1565
10	100%	84%	114%	106%
20	103%	95%	116%	113%
40	89%	91%	116%	91%

IL-29				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	14	555	27	259
1:10	7.1	303.0	15.8	136.0
1:20	4.1	143.5	7.7	66.5
1:40	2.3	77.4	3.7	33.8
10	101%	109%	116%	105%
20	116%	103%	113%	103%
40	130%	112%	108%	104%

IL-31				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	165	5575	170	90
1:10	88	2740	109	54
1:20	50	1350	53	25
1:40	25	671	31	<LLOQ
10	107%	98%	128%	120%
20	122%	97%	125%	112%
40	120%	96%	145%	<LLOQ

IL-9				
pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
1:5	132	2110	444	659
1:10	79	1145	205	320
1:20	42	503	104	172
1:40	20	273	56	97
10	120%	109%	92%	97%
20	129%	95%	93%	104%
40	124%	104%	100%	118%

6. **Freeze/thaw stability:** Samples were assessed for freeze-thaw stability after 3 F/T cycles. All values were within the acceptance range of 80-120% for freeze-thaw samples compared to the non-freeze thawed samples indicating that samples could be freeze-thawed up to 3 times without a loss in signal.

	GM-CSF				
	pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	FT-0X	75	1500	140	1038
	FT-1X	84	1630	137	990
	FT-2X	82	1675	145	1055
	FT-3X	71	1635	152	1030
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	112%	109%	98%	95%
	FT-2X	108%	112%	104%	102%
	FT-3X	94%	109%	109%	99%

	IFNα				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	7	551	12	240
	FT-1X	7	481	12	234
	FT-2X	8	540	12	239
	FT-3X	7	444	14	245
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	103%	87%	105%	97%
	FT-2X	107%	98%	99%	100%
	FT-3X	101%	81%	120%	102%

	IFNβ				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	333	1465	287	940
	FT-1X	367	1255	331	894
	FT-2X	362	1455	326	895
	FT-3X	346	1435	355	947
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	110%	86%	116%	95%
	FT-2X	109%	99%	114%	95%
	FT-3X	104%	98%	124%	101%

	IL-12p70				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	147	2850	211	1490
	FT-1X	161	2365	215	1455
	FT-2X	151	2795	208	1325
	FT-3X	139	2255	215	1395
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	109%	83%	102%	98%
	FT-2X	103%	98%	99%	89%
	FT-3X	94%	79%	102%	94%

	IL-17				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	46	903	60	473
	FT-1X	48	765	63	476
	FT-2X	44	841	62	479
	FT-3X	44	750	65	488
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	104%	85%	105%	101%
	FT-2X	96%	93%	105%	101%
	FT-3X	95%	83%	109%	103%

	IL-21				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	205	3250	264	1020
	FT-1X	227	2875	271	1030
	FT-2X	204	3205	266	1010
	FT-3X	203	2545	277	1030
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	110%	88%	102%	101%
	FT-2X	99%	99%	101%	99%
	FT-3X	99%	78%	105%	101%

	IL-22				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	519	2680	124	1150
	FT-1X	501	2030	132	1105
	FT-2X	498	2330	128	1115
	FT-3X	481	1910	125	996
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	97%	76%	107%	96%
	FT-2X	96%	87%	103%	97%
	FT-3X	93%	71%	101%	87%

	IL-23				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	478	5050	1650	10035
	FT-1X	540	3870	1670	9180
	FT-2X	511	4695	1640	9650
	FT-3X	523	4045	1665	9515
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	113%	77%	101%	91%
	FT-2X	107%	93%	99%	96%
	FT-3X	109%	80%	101%	95%

	IL-27				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	762	15300	2010	16650
	FT-1X	808	11700	2200	14050
	FT-2X	788	14350	2295	14700
	FT-3X	742	13200	2275	14050
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	106%	76%	109%	84%
	FT-2X	103%	94%	114%	88%
	FT-3X	97%	86%	113%	84%

	IL-29				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	16	592	28	285
	FT-1X	16	480	31	284
	FT-2X	17	566	29	287
	FT-3X	15	462	30	287
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	100%	81%	110%	100%
	FT-2X	107%	96%	105%	101%
	FT-3X	95%	78%	107%	101%

	IL-31				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	151	2370	205	1270
	FT-1X	162	2090	189	1295
	FT-2X	170	2320	213	1260
	FT-3X	156	1965	214	1265
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	108%	88%	92%	102%
	FT-2X	113%	98%	104%	99%
	FT-3X	104%	83%	105%	100%

	IL-9				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	134	2400	175	825
	FT-1X	144	2050	186	829
	FT-2X	144	2260	190	817
	FT-3X	139	1880	201	825
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	108%	85%	106%	100%
	FT-2X	107%	94%	109%	99%
	FT-3X	104%	78%	115%	100%

7. **Bench Top Stability:** Samples were assessed for bench top stability at 2hr RT to determine if the samples were stable on the bench prior to the assay or if refrigeration was required. All values were within the acceptance range of 80-120% for samples compared to the bench top samples indicating that no loss in activity will occur during the testing of the samples.

	GM-CSF				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	103	1440	158	1011
	2hr RT	89	1650	142	1085
	2hr 4C	89	1550	148	1009
	4hr 4C	94	1610	143	908
% Control	0 HR	100%	100%	100%	100%
	2hr RT	87%	115%	90%	107%
	2hr 4C	87%	108%	94%	100%
	4hr 4C	92%	112%	90%	90%

	IFNα				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	11	598	12	229
	2hr RT	10	619	12	226
	2hr 4C	10	606	12	220
	4hr 4C	10	554	12	191
% Control	0 HR	100%	100%	100%	100%
	2hr RT	94%	104%	102%	99%
	2hr 4C	89%	101%	98%	96%
	4hr 4C	90%	93%	103%	83%

	IFNβ				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	462	1755	352	1105
	2hr RT	490	1725	341	1160
	2hr 4C	442	1735	375	1070
	4hr 4C	469	1550	344	973
% Control	0 HR	100%	100%	100%	100%
	2hr RT	106%	98%	97%	105%
	2hr 4C	96%	99%	106%	97%
	4hr 4C	101%	88%	98%	88%

	IL-12p70				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	155	2485	192	1290
	2hr RT	138	2545	172	1390
	2hr 4C	155	2585	198	1180
	4hr 4C	160	2270	172	1245
% Control	0 HR	100%	100%	100%	100%
	2hr RT	89%	102%	90%	108%
	2hr 4C	100%	104%	103%	91%
	4hr 4C	103%	91%	90%	97%

	IL-17				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	53	855	52	443
	2hr RT	51	997	53	442
	2hr 4C	49	860	59	448
	4hr 4C	51	801	58	519
% Control	0 HR	100%	100%	100%	100%
	2hr RT	97%	117%	102%	100%
	2hr 4C	93%	101%	113%	101%
	4hr 4C	96%	94%	111%	117%

	IL-21				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	244	3040	261	1030
	2hr RT	232	3215	241	1019
	2hr 4C	212	3245	267	980
	4hr 4C	211	2805	267	802
% Control	0 HR	100%	100%	100%	100%
	2hr RT	95%	106%	92%	99%
	2hr 4C	87%	107%	102%	95%
	4hr 4C	86%	92%	102%	78%

	IL-22				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	574	2720	117	939
	2hr RT	475	2220	99	826
	2hr 4C	551	2500	122	944
	4hr 4C	516	2135	110	799
% Control	0 HR	100%	100%	100%	100%
	2hr RT	83%	82%	85%	88%
	2hr 4C	96%	92%	104%	100%
	4hr 4C	90%	78%	94%	85%

	IL-23				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	627	5345	1370	8500
	2hr RT	624	4325	1350	8315
	2hr 4C	639	5005	1410	8115
	4hr 4C	621	4590	1365	8145
% Control	0 HR	100%	100%	100%	100%
	2hr RT	99%	81%	99%	98%
	2hr 4C	102%	94%	103%	95%
	4hr 4C	99%	86%	100%	96%

	IL-27				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	861	13650	1985	13350
	2hr RT	766	12100	1925	12500
	2hr 4C	837	14150	2085	12300
	4hr 4C	843	11750	1935	13200
% Control	0 HR	100%	100%	100%	100%
	2hr RT	89%	89%	97%	94%
	2hr 4C	97%	104%	105%	92%
	4hr 4C	98%	86%	97%	99%

	IL-29				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	21	527	27	251
	2hr RT	18	474	27	235
	2hr 4C	17	499	29	231
	4hr 4C	19	427	27	222
% Control	0 HR	100%	100%	100%	100%
	2hr RT	89%	90%	98%	94%
	2hr 4C	83%	95%	107%	92%
	4hr 4C	93%	81%	101%	88%

	IL-31				
	pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	194	2445	209	1370
	2hr RT	187	2405	221	1470
	2hr 4C	175	2505	214	1320
	4hr 4C	176	2330	207	1190
% Control	0 HR	100%	100%	100%	100%
	2hr RT	96%	98%	106%	107%
	2hr 4C	90%	102%	102%	96%
	4hr 4C	91%	95%	99%	87%

	IL-9				
	pg/ml	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	0 HR	163	2070	178	753
	2hr RT	159	2045	172	809
	2hr 4C	162	2080	180	752
	4hr 4C	163	1925	174	751
% Control	0 HR	100%	100%	100%	100%
	2hr RT	98%	99%	97%	107%
	2hr 4C	100%	100%	101%	100%
	4hr 4C	100%	93%	98%	100%