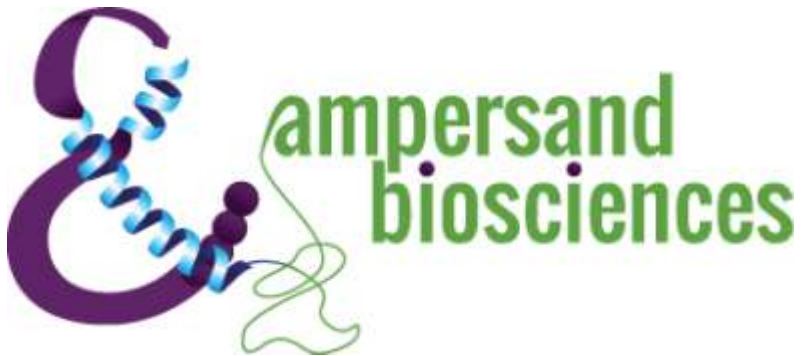


Mouse Cytokine Panel 3

Kit # M108-K

Validation Report Version 1.0

May 24, 2024



1. Assay Description:

A multiplex assay was developed and validated for the measurement of Mouse GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF. 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 μ L of standard, controls or sample are added to the appropriate wells, followed by 10 μ L of blocker and 10 μ L of multiplexed capture-antibody microspheres. The plate is incubated for 1 hour at ambient temperature on a plate shaker. After washing 3 times, 40 μ L 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 μ L of wash buffer. After shaking on a plate shaker for 5 minutes, the plate is then analyzed on the Luminex 200 Analyzer.

2. Control and Sample Description:

Control	Description
Control 1	Normal Mouse Serum (20%) spiked with low levels of Recombinant GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF.
Control 2	Normal Mouse Serum (20%) spiked with Recombinant GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF.

Sample	Description
Serum 1	Normal Mouse Serum (50%) spiked with Recombinant GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF.
Serum 2	Normal Mouse Serum (50%) spiked with Recombinant GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF.
Plasma 1	Normal Mouse Plasma (50%) spiked with Recombinant GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF.
Plasma 2	Normal Mouse Plasma (50%) spiked with Recombinant GM-CSF, IFN- β , IL-17, IL-18, IL-22, IL-23, IL-27, IL-28, IL-9, MIP-2, TSLP and VEGF.

3. LLOQ, LDD and Curves:

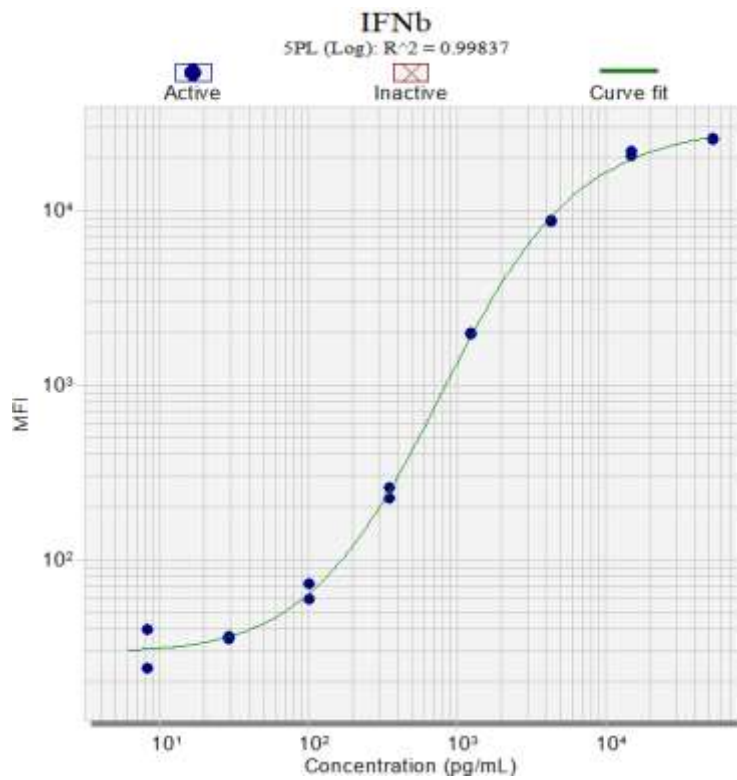
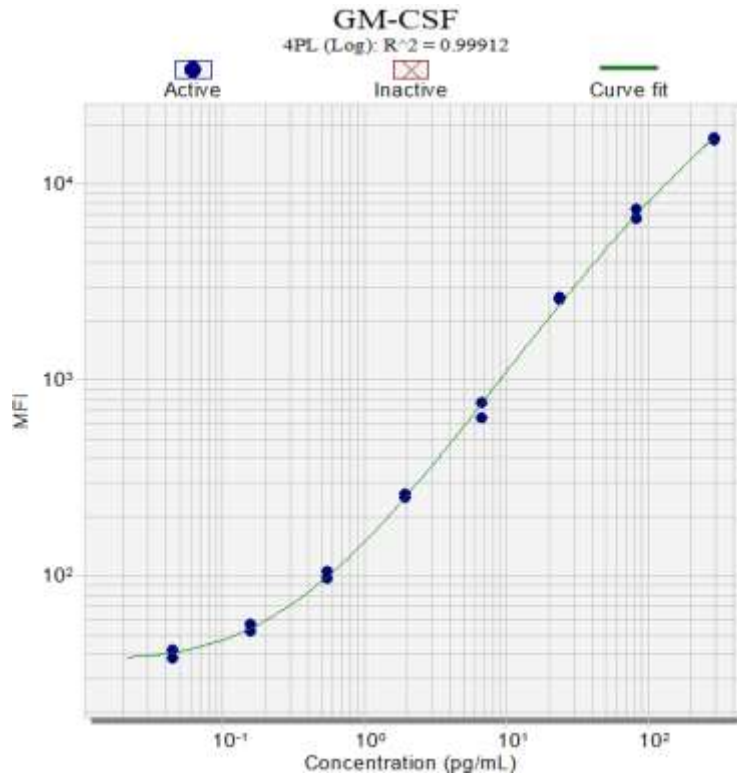
LDD: MFI (Median Fluorescent Intensity) for 20 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

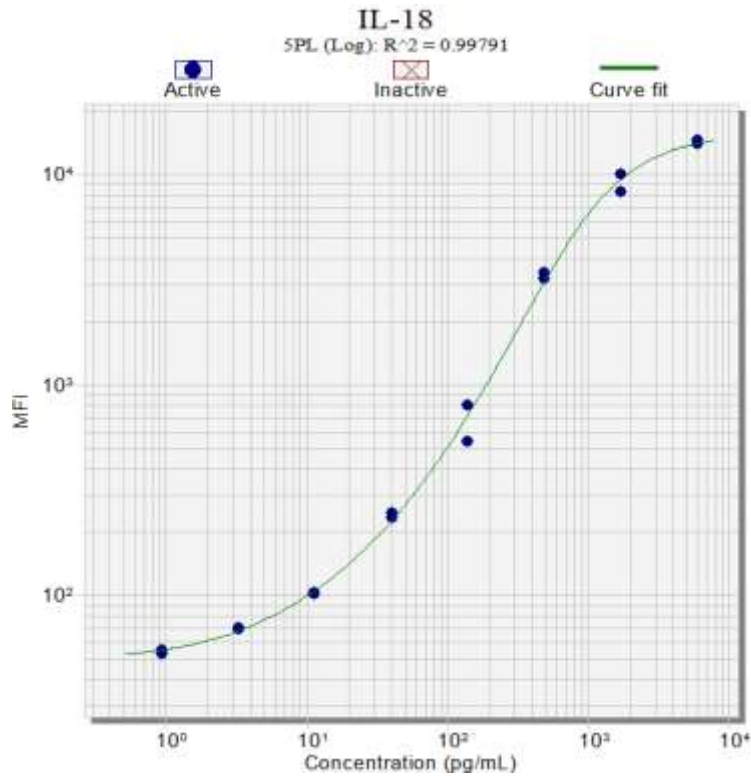
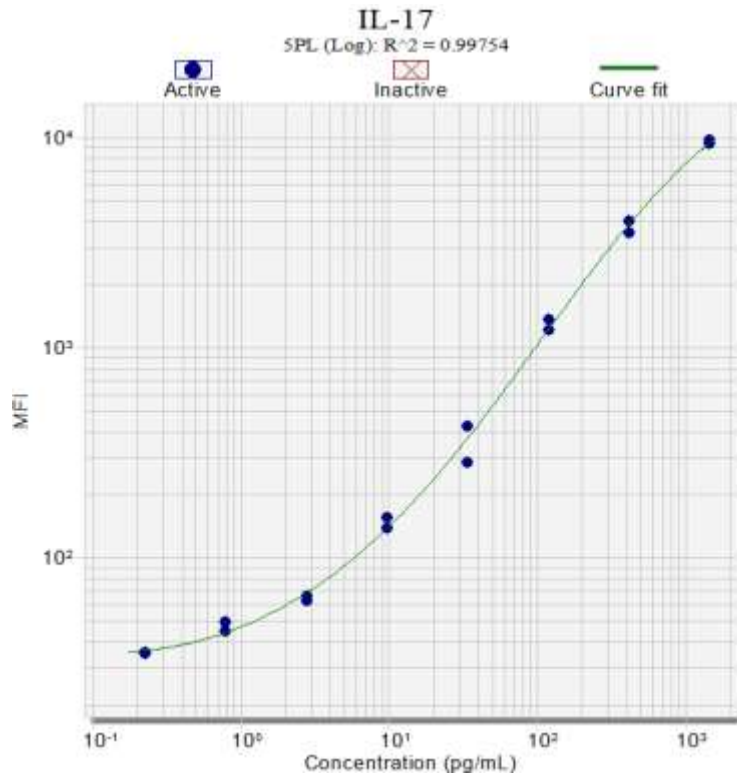
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in triplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

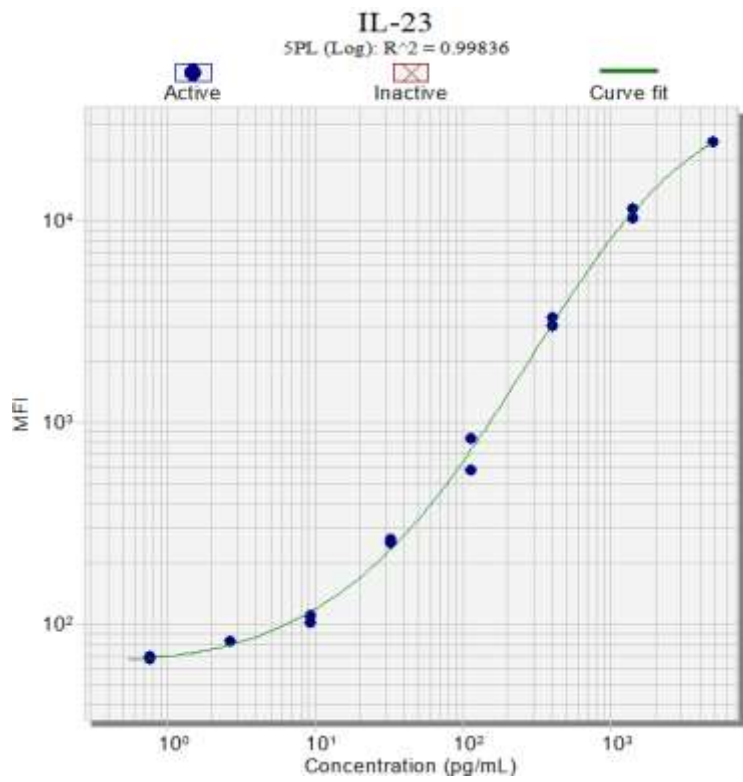
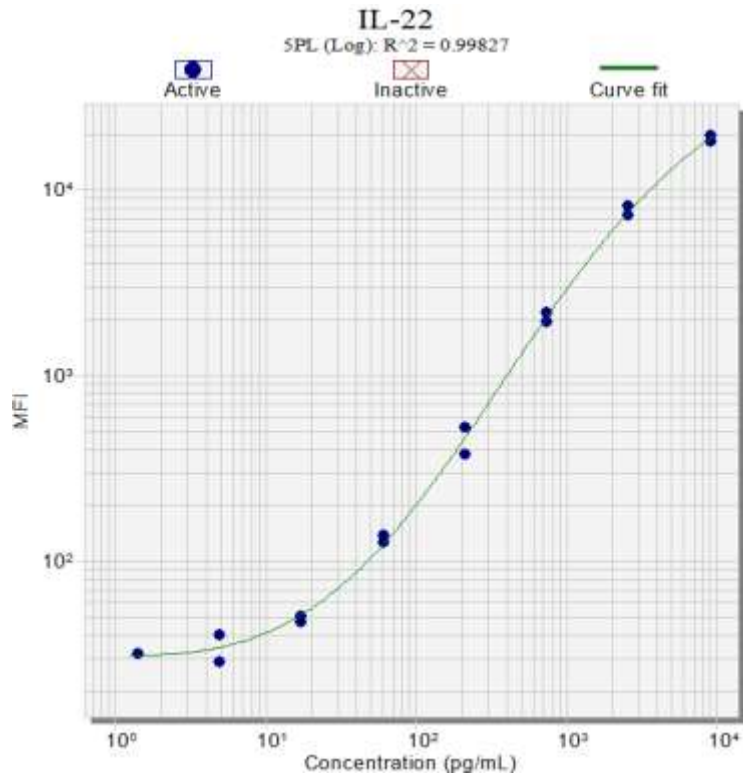
Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.

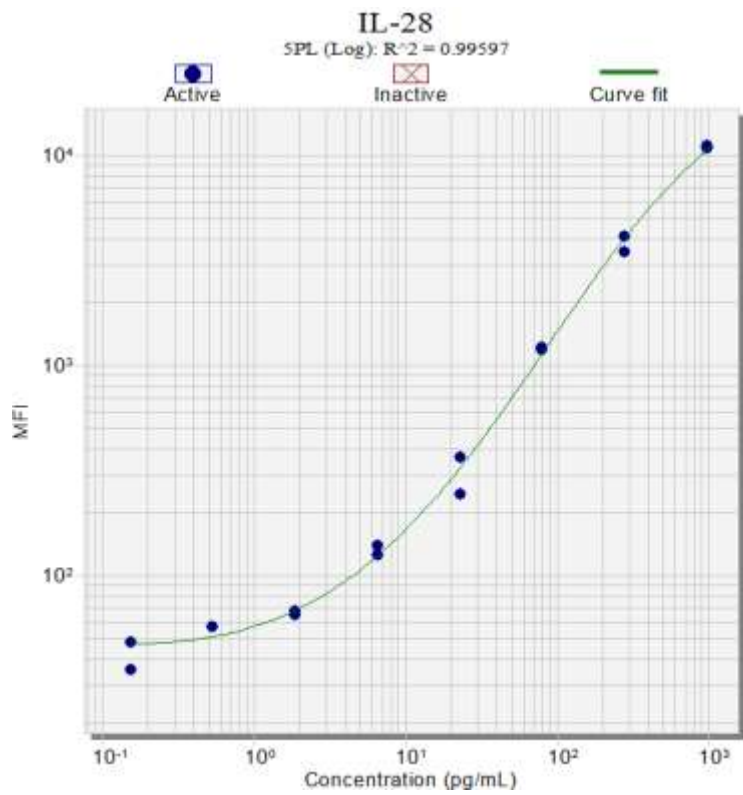
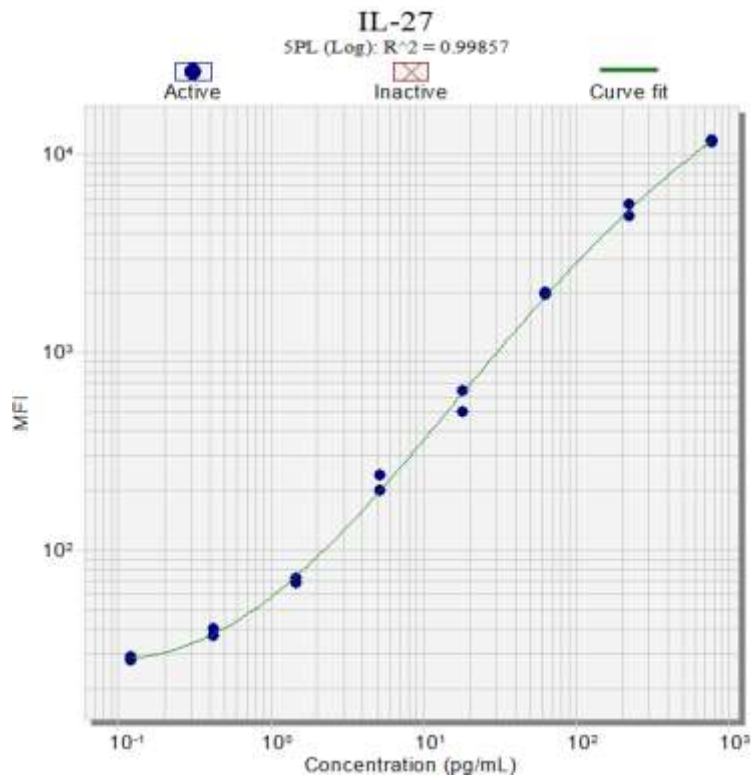
		S1	S8	LDD	LLOQ
GM-CSF	pg/mL	0.045	290	0.24	0.44
IFNβ	pg/mL	8.3	53275	43	63
IL-17	pg/mL	0.23	1460	1.1	3.5
IL-18	pg/mL	0.93	5970	3.9	5.6
IL-22	pg/mL	1.4	9000	8.0	17
IL-23	pg/mL	0.76	4885	5.9	9.0
IL-27	pg/mL	0.12	773	0.24	0.67
IL-28	pg/mL	0.15	975	1.8	2.9
IL-9	pg/mL	3.1	19860	39	76
MIP-2	pg/mL	0.13	800	0.46	1.3
TSLP	pg/mL	0.11	715	0.16	0.66
VEGF	pg/mL	0.17	1100	0.38	2.3

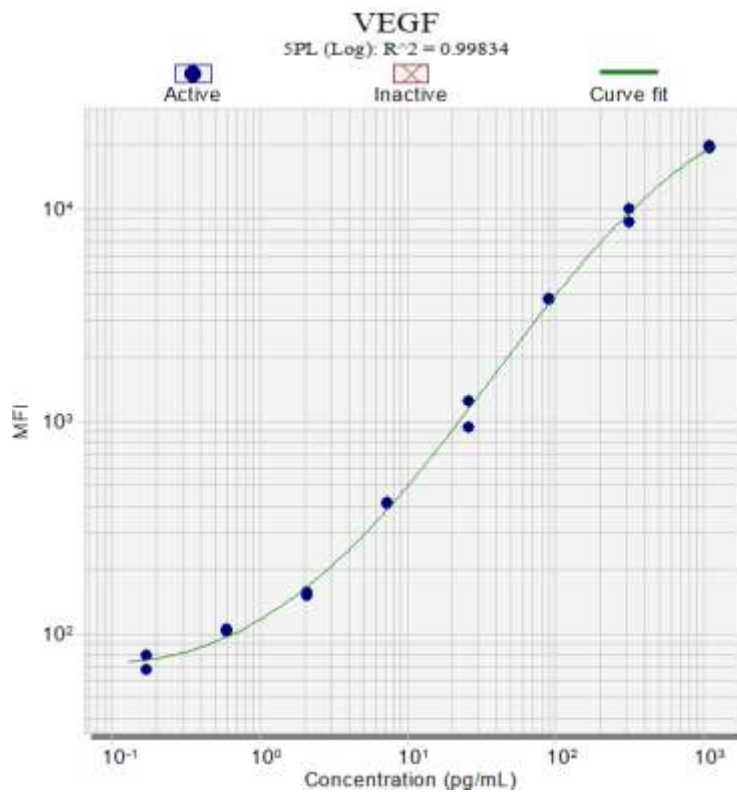
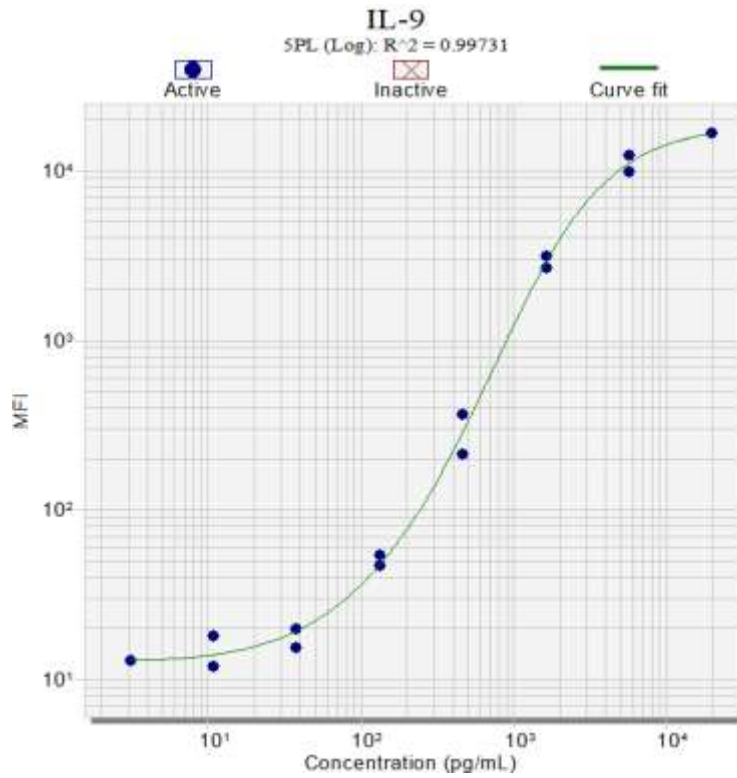
X Dilution Factor		S1	S8	LDD	LLOQ
GM-CSF	pg/mL	0.22	1450	1.2	2.2
IFNβ	pg/mL	41	266375	213	315
IL-17	pg/mL	1.1	7300	5.7	18
IL-18	pg/mL	4.7	29850	20	28
IL-22	pg/mL	7.0	45000	40	85
IL-23	pg/mL	3.8	24425	29	45
IL-27	pg/mL	0.60	3865	1.2	3.4
IL-28	pg/mL	0.76	4875	9.0	15
IL-9	pg/mL	15	99300	193	380
MIP-2	pg/mL	0.63	4000	2.3	6.5
TSLP	pg/mL	0.56	3575	0.82	3.3
VEGF	pg/mL	0.86	5500	1.9	12

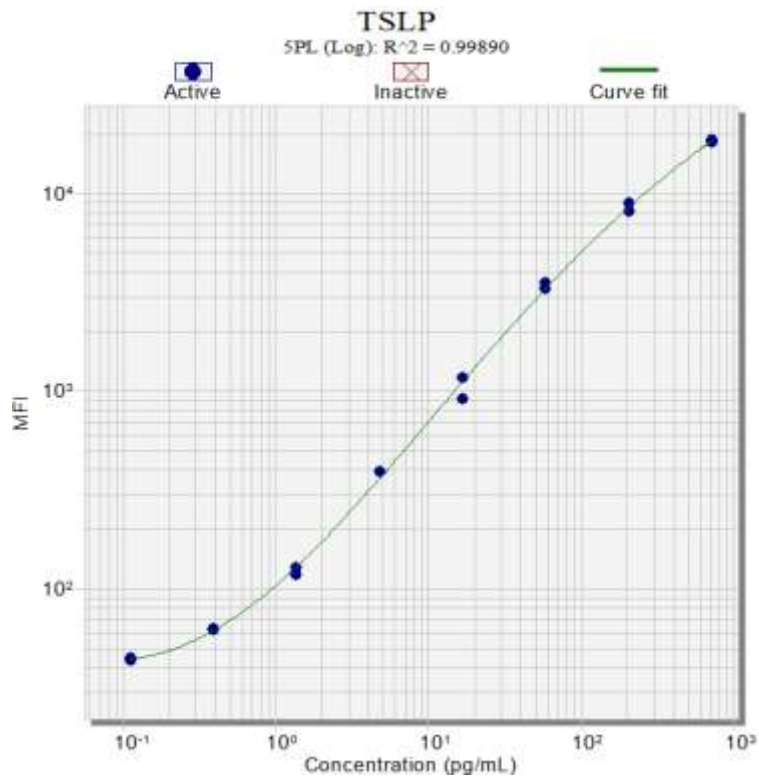
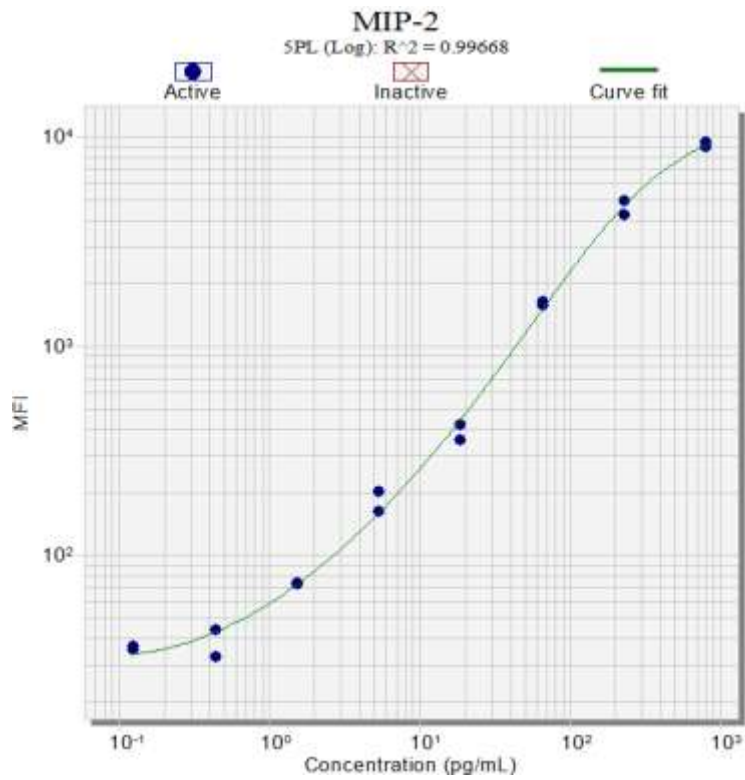












4. Precision:

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	2.9	2.6	2.7	2.8
	% CV	9%	8%	3%	8%
Control 2	Mean	37	33	38	36
	% CV	8%	1%	7%	9%

IFNb		1	2	3	Inter
Control 1	Mean	317	337	308	318
	% CV	6%	12%	9%	8%
Control 2	Mean	2903	3215	2630	2879
	% CV	2%	0%	2%	9%

IL-17		1	2	3	Inter
Control 1	Mean	69	68	68	69
	% CV	5%	6%	3%	4%
Control 2	Mean	1027	1150	941	1026
	% CV	6%	4%	3%	9%

IL-18		1	2	3	Inter
Control 1	Mean	115	98	121	113
	% CV	4%	8%	9%	11%
Control 2	Mean	1004	741	898	898
	% CV	5%	7%	2%	13%

IL-22		1	2	3	Inter
Control 1	Mean	148	151	169	156
	% CV	2%	0%	3%	7%
Control 2	Mean	1270	1185	1260	1245
	% CV	3%	4%	2%	4%

IL-23		1	2	3	Inter
Control 1	Mean	69	75	72	72
	% CV	7%	16%	3%	8%
Control 2	Mean	560	546	588	567
	% CV	5%	2%	1%	4%

IL-27		1	2	3	Inter
Control 1	Mean	22	22	23	23
	% CV	6%	6%	3%	5%
Control 2	Mean	367	358	353	360
	% CV	3%	1%	2%	3%

IL-28		1	2	3	Inter
Control 1	Mean	38	33	37	36
	% CV	4%	6%	6%	7%
Control 2	Mean	381	320	343	352
	% CV	2%	3%	7%	8%

IL-9		1	2	3	Inter
Control 1	Mean	592	521	601	578
	% CV	6%	5%	4%	7%
Control 2	Mean	2837	2610	2763	2753
	% CV	3%	3%	5%	5%

MIP-2		1	2	3	Inter
Control 1	Mean	44	37	40	41
	% CV	4%	6%	5%	9%
Control 2	Mean	489	469	408	454
	% CV	6%	7%	7%	10%

TSLP		1	2	3	Inter
Control 1	Mean	18	17	17	17
	% CV	8%	5%	7%	7%
Control 2	Mean	209	201	217	210
	% CV	5%	4%	4%	5%

VEGF		1	2	3	Inter
Control 1	Mean	6.1	5.7	6.0	6.0
	% CV	13%	12%	5%	9%
Control 2	Mean	74	68	77	73
	% CV	5%	2%	9%	8%

5. Linearity:

Linearity was assessed using 2 serum and 2 plasma 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
1:2	42	18	133	11
1:4	21	8.3	68	4.9
1:8	12	4.4	40	2.0
1:16	6.1	2.2	17	1.0
2	100%	93%	103%	92%
4	116%	99%	120%	76%
8	115%	98%	104%	72%

IFNβ				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	7815	1993	27400	2370
1:4	3805	936	12150	1150
1:8	1905	422	6465	609
1:16	993	286	2815	313
2	97%	94%	89%	97%
4	98%	85%	94%	103%
8	102%	115%	82%	105%

IL-17				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	218	200	763	62
1:4	85	114	316	28
1:8	53	59	175	13
1:16	30	32	77	8.8
2	78%	114%	83%	90%
4	97%	119%	91%	85%
8	109%	127%	81%	113%

IL-18				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	606	390	898	1200
1:4	299	210	581	591
1:8	159	117	273	382
1:16	98	47	139	190
2	99%	108%	129%	98%
4	105%	120%	121%	127%
8	130%	96%	123%	127%

IL-22				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	1325	538	4235	312
1:4	695	255	2065	138
1:8	382	120	1095	78
1:16	192	63	462	31
2	105%	95%	98%	88%
4	115%	89%	103%	100%
8	116%	93%	87%	78%

IL-23				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	738	214	2205	718
1:4	380	89	1140	335
1:8	212	46	422	194
1:16	102	33	255	109
2	103%	83%	103%	93%
4	115%	86%	77%	108%
8	110%	123%	93%	121%

IL-27				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	53	91	345	20
1:4	33	41	189	10
1:8	13	20	98	5.3
1:16	6.5	8.1	38	2.7
2	123%	91%	110%	100%
4	101%	90%	114%	108%
8	98%	72%	87%	111%

IL-28				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	148	219	486	44
1:4	82	109	240	23
1:8	42	52	136	14
1:16	22	26	57	8.8
2	112%	100%	99%	103%
4	113%	96%	112%	124%
8	121%	96%	95%	<LLOQ

IL-9				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	2975	1530	9495	915
1:4	1695	731	4780	460
1:8	854	315	2550	228
1:16	439	265	1355	145
2	114%	96%	101%	101%
4	115%	82%	107%	100%
8	118%	<LLOQ	114%	126%

MIP-2				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	151	80	463	36
1:4	72	41	218	20
1:8	42	15	114	11
1:16	18	9.1	44	4.9
2	95%	103%	94%	114%
4	111%	74%	98%	123%
8	96%	91%	76%	110%

TSLP				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	111	67	351	32
1:4	56	31	183	16
1:8	29	15	103	9.2
1:16	13	8.7	40	4.7
2	100%	93%	104%	101%
4	105%	92%	117%	116%
8	92%	105%	90%	118%

VEGF				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	143	35	244	40
1:4	69	17	139	18
1:8	42	9.1	58	12
1:16	20	5.1	34	6.1
2	97%	97%	114%	87%
4	118%	103%	95%	122%
8	111%	115%	111%	120%

6. **Freeze/thaw stability:** Samples were assessed for freeze-thaw stability after 1, 2, and 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		50	20	174	14
	FT-1X		52	17	164	13
	FT-2X		48	18	182	15
	FT-3X		52	18	176	15
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		103%	87%	94%	94%
	FT-2X		96%	90%	105%	108%
	FT-3X		104%	91%	101%	107%

		IFN β				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		12350	2735	47500	5280
	FT-1X		11400	2630	45850	4945
	FT-2X		11500	2595	50150	5105
	FT-3X		12050	2630	43200	5355
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		92%	96%	97%	94%
	FT-2X		93%	95%	106%	97%
	FT-3X		98%	96%	91%	101%

		IL-17				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		242	604	1665	144
	FT-1X		217	471	1480	126
	FT-2X		220	510	1745	140
	FT-3X		243	521	1645	140
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		90%	78%	89%	88%
	FT-2X		91%	84%	105%	97%
	FT-3X		100%	86%	99%	97%

		IL-18			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	649	708	1925	208
	FT-1X	607	599	1705	188
	FT-2X	567	645	1785	201
	FT-3X	632	604	1820	227
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	94%	85%	89%	90%
	FT-2X	87%	91%	93%	97%
	FT-3X	97%	85%	95%	109%

		IL-22			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	2550	1020	8345	1023
	FT-1X	2340	861	7730	969
	FT-2X	2325	972	8625	1006
	FT-3X	2495	938	8050	1019
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	92%	84%	93%	95%
	FT-2X	91%	95%	103%	98%
	FT-3X	98%	92%	96%	100%

		IL-23			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	1810	507	5755	640
	FT-1X	1780	412	5465	723
	FT-2X	1795	459	6090	714
	FT-3X	1840	455	5725	738
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	98%	81%	95%	113%
	FT-2X	99%	90%	106%	112%
	FT-3X	102%	90%	99%	115%

		IL-27			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	264	189	917	75
	FT-1X	239	153	801	68
	FT-2X	246	170	951	68
	FT-3X	260	161	825	82
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	91%	81%	87%	92%
	FT-2X	93%	90%	104%	91%
	FT-3X	99%	85%	90%	110%

	IL-28				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	197	238	667	66
	FT-1X	182	202	611	62
	FT-2X	191	212	730	70
	FT-3X	199	208	662	71
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	93%	85%	92%	93%
	FT-2X	97%	89%	109%	105%
	FT-3X	101%	87%	99%	107%

	IL-9				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	3845	1790	11700	1010
	FT-1X	3490	1540	10720	1055
	FT-2X	3660	1625	12400	1110
	FT-3X	3760	1590	11350	1038
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	91%	86%	92%	104%
	FT-2X	95%	91%	106%	110%
	FT-3X	98%	89%	97%	103%

	MIP-2				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	258	291	951	87
	FT-1X	242	232	894	77
	FT-2X	237	259	978	77
	FT-3X	262	254	930	72
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	94%	80%	94%	88%
	FT-2X	92%	89%	103%	89%
	FT-3X	101%	87%	98%	83%

	TSLP				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	210	127	649	58
	FT-1X	201	102	606	53
	FT-2X	199	104	701	56
	FT-3X	205	111	648	59
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	96%	80%	93%	92%
	FT-2X	95%	82%	108%	98%
	FT-3X	98%	88%	100%	102%

		VEGF			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	206	37	605	38
	FT-1X	192	29	578	35
	FT-2X	192	29	669	37
	FT-3X	207	26	618	42
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	93%	79%	96%	90%
	FT-2X	93%	80%	111%	97%
	FT-3X	101%	72%	102%	110%

7. Bench Top Stability: Samples were assessed bench top stability and 2hr RT, and 2 & 4hr 4°C 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	CTL-0 Hr	41	14	158	15
	2hr RT	53	17	167	12
	2hr 4C	49	18	194	16
	4hr 4C	45	12	161	15
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	129%	118%	106%	82%
	2hr 4C	120%	125%	122%	108%
	4hr 4C	109%	88%	102%	105%

		IFN β			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	11900	2485	43750	5460
	2hr RT	12800	2820	43000	4560
	2hr 4C	12300	2530	55600	5595
	4hr 4C	12150	2200	45550	5250
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	108%	113%	98%	84%
	2hr 4C	103%	102%	127%	102%
	4hr 4C	102%	89%	104%	96%

		IL-17			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	199	421	1370	117
	2hr RT	228	348	1335	138
	2hr 4C	211	428	1730	138
	4hr 4C	210	296	1335	98
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	114%	83%	97%	118%
	2hr 4C	106%	102%	126%	118%
	4hr 4C	106%	70%	97%	84%

		IL-18			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	649	584	1705	265
	2hr RT	719	668	1605	313
	2hr 4C	658	604	1945	254
	4hr 4C	618	435	1630	210
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	111%	114%	94%	118%
	2hr 4C	101%	103%	114%	96%
	4hr 4C	95%	75%	96%	79%

		IL-22			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	2520	868	8265	1080
	2hr RT	2810	878	7985	849
	2hr 4C	2600	917	9315	1100
	4hr 4C	2380	804	8295	1013
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	112%	101%	97%	79%
	2hr 4C	103%	106%	113%	102%
	4hr 4C	94%	93%	100%	94%

		IL-23			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	1795	389	5650	758
	2hr RT	1965	448	5780	658
	2hr 4C	2075	449	6710	817
	4hr 4C	1885	353	6225	763
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	109%	115%	102%	87%
	2hr 4C	116%	115%	119%	108%
	4hr 4C	105%	91%	110%	101%

		IL-27			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	244	139	859	74
	2hr RT	266	165	813	60
	2hr 4C	268	155	1080	77
	4hr 4C	239	126	867	72
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	109%	118%	95%	81%
	2hr 4C	110%	111%	126%	104%
	4hr 4C	98%	90%	101%	97%

		IL-28			
		ng/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	176	189	604	74
	2hr RT	197	192	594	59
	2hr 4C	202	198	729	83
	4hr 4C	212	152	625	77
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	112%	101%	98%	79%
	2hr 4C	114%	104%	121%	112%
	4hr 4C	120%	80%	103%	104%

		IL-9			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	3405	1510	11100	1180
	2hr RT	4015	1655	11050	856
	2hr 4C	3825	1585	12400	1145
	4hr 4C	3740	1320	10800	1185
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	118%	110%	100%	73%
	2hr 4C	112%	105%	112%	97%
	4hr 4C	110%	87%	97%	100%

		MIP-2			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	218	202	777	81
	2hr RT	250	204	781	57
	2hr 4C	234	215	1000	94
	4hr 4C	228	153	784	81
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	114%	101%	101%	70%
	2hr 4C	107%	106%	129%	116%
	4hr 4C	104%	76%	101%	100%

		TSLP				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr		185	93	607	62
	2hr RT		219	102	658	47
	2hr 4C		208	99	741	64
	4hr 4C		197	82	636	60
% Control	CTL-0 Hr		100%	100%	100%	100%
	2hr RT		118%	109%	108%	77%
	2hr 4C		112%	106%	122%	103%
	4hr 4C		106%	88%	105%	98%

		VEGF				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr		185	27	571	38
	2hr RT		200	28	587	35
	2hr 4C		202	27	725	41
	4hr 4C		182	20	609	31
% Control	CTL-0 Hr		100%	100%	100%	100%
	2hr RT		108%	103%	103%	91%
	2hr 4C		109%	99%	127%	108%
	4hr 4C		99%	74%	107%	83%