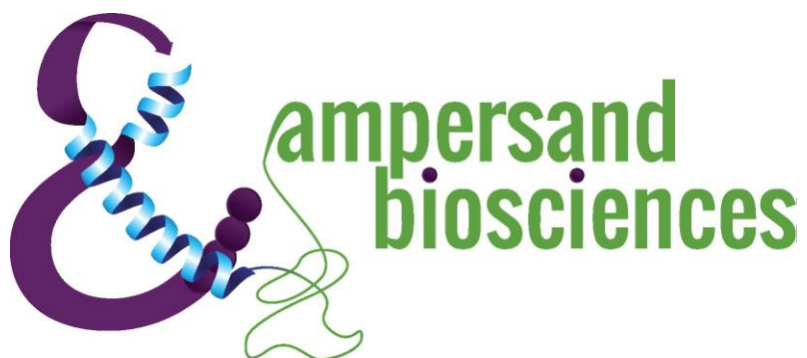


Ferret Cytokine Panel 1

Kit # F103-K

Validation Report



Prepared by: _____

Date _____

Reviewed by: _____

Date _____

1. Assay Description:

A multiplex assay was developed and validated for the measurement of Ferret IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1 β , and TNF α . 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 2 hours 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 Ferret Serum (33%) spiked with Recombinant IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1 β , and TNF α .
Control 2	Normal Ferret Serum (33%) spiked with Recombinant IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1 β , and TNF α .

Sample	Description
Serum 1	Normal Ferret Serum spiked with Recombinant IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, MIP-1 β and TNF α .
Serum 2	Normal Ferret Serum spiked with Recombinant IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1 β , and TNF α .
Plasma 1	Normal Ferret Plasma spiked with Recombinant IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, MCP-1, MIP-1 β , and TNF α .
Plasma 2	Normal Ferret Serum spiked with Recombinant IFN γ , IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, MIP-1 β , and TNF α .

3. LLOQ, LDD and Curves:

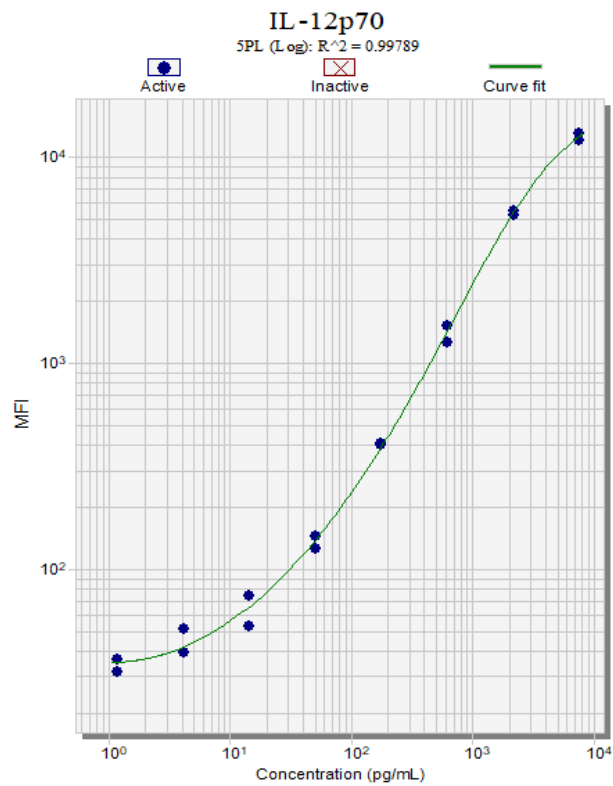
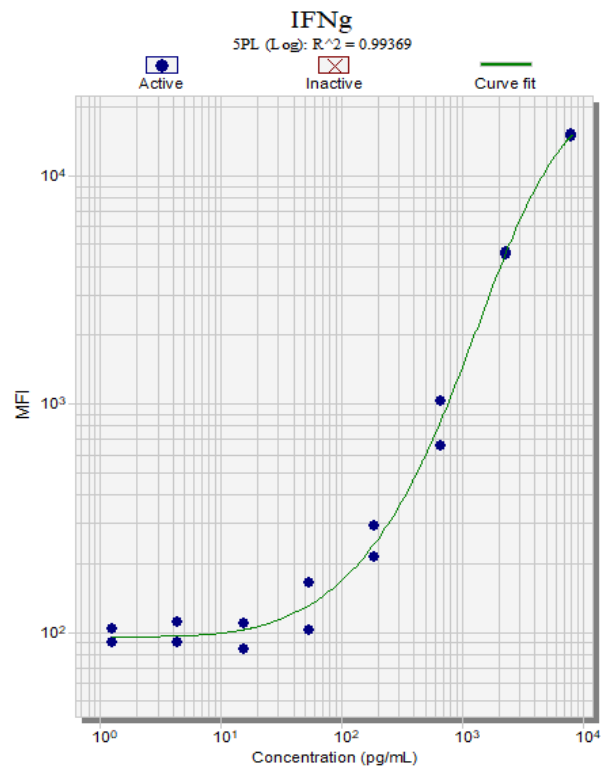
LDD: MFI (Median Fluorescent Intensity) for 20 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

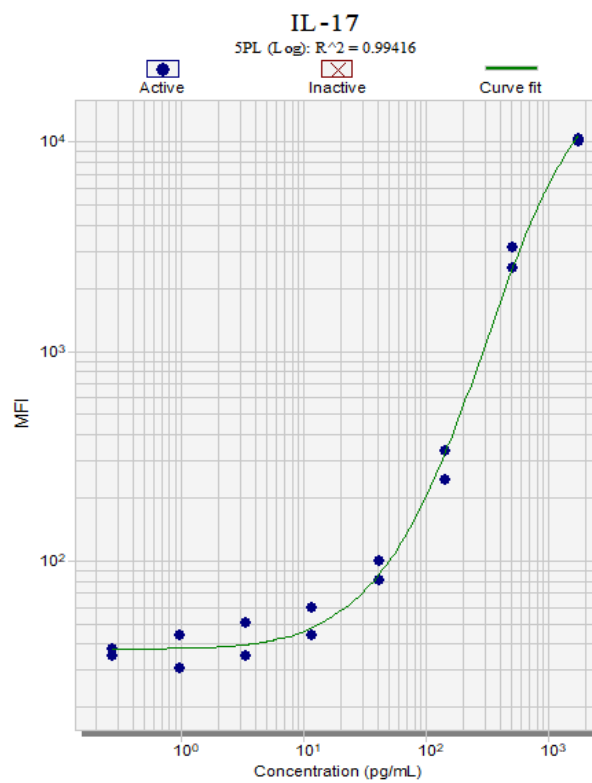
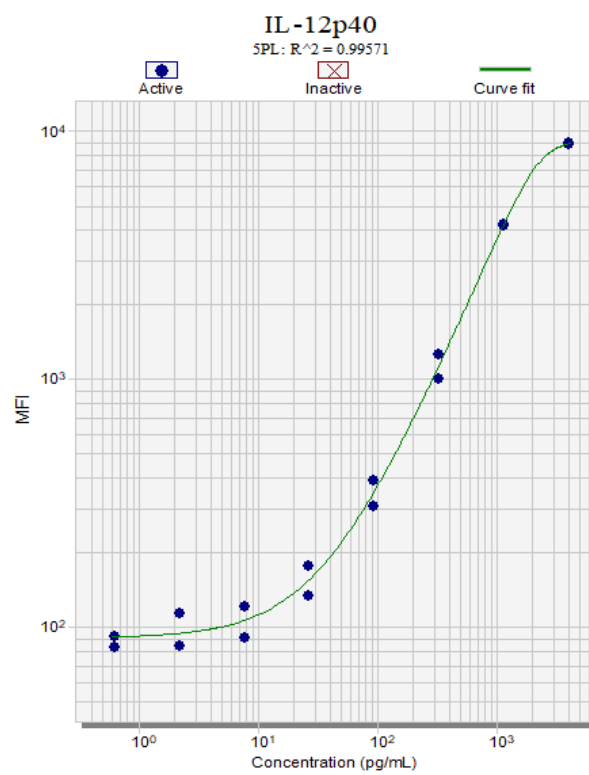
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in triplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

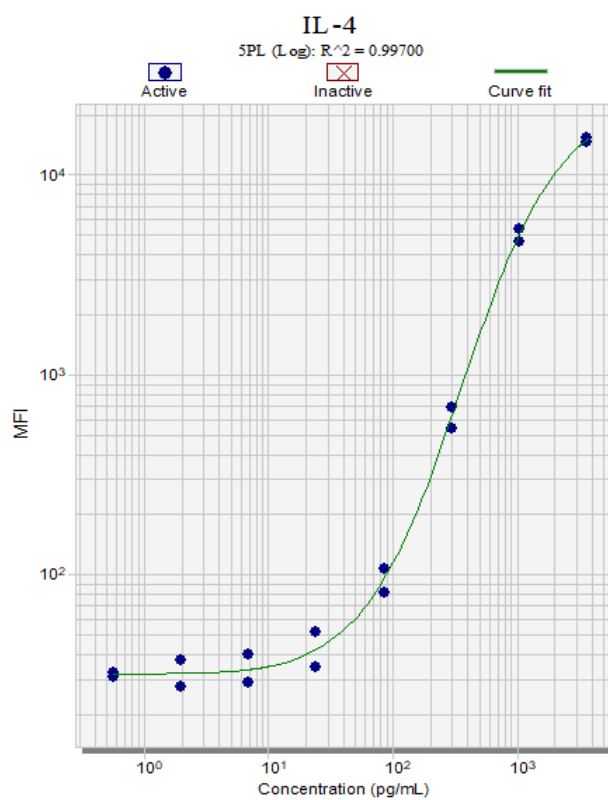
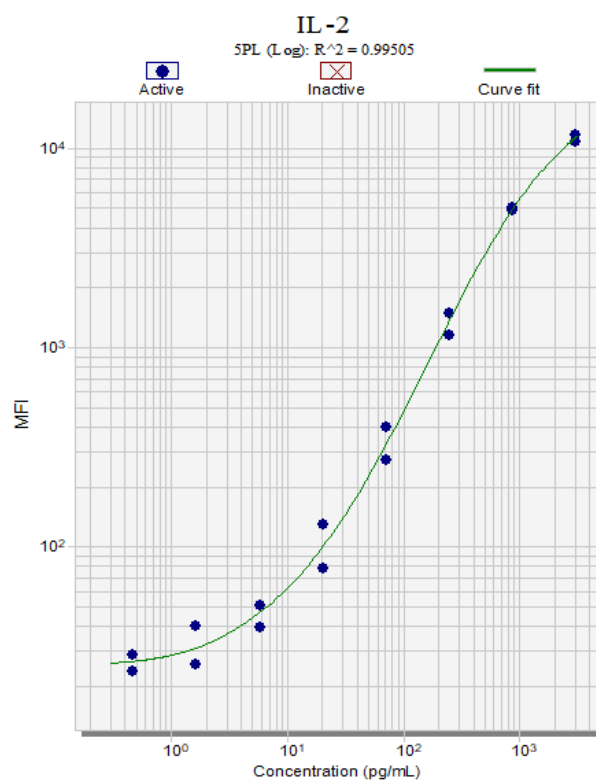
Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.

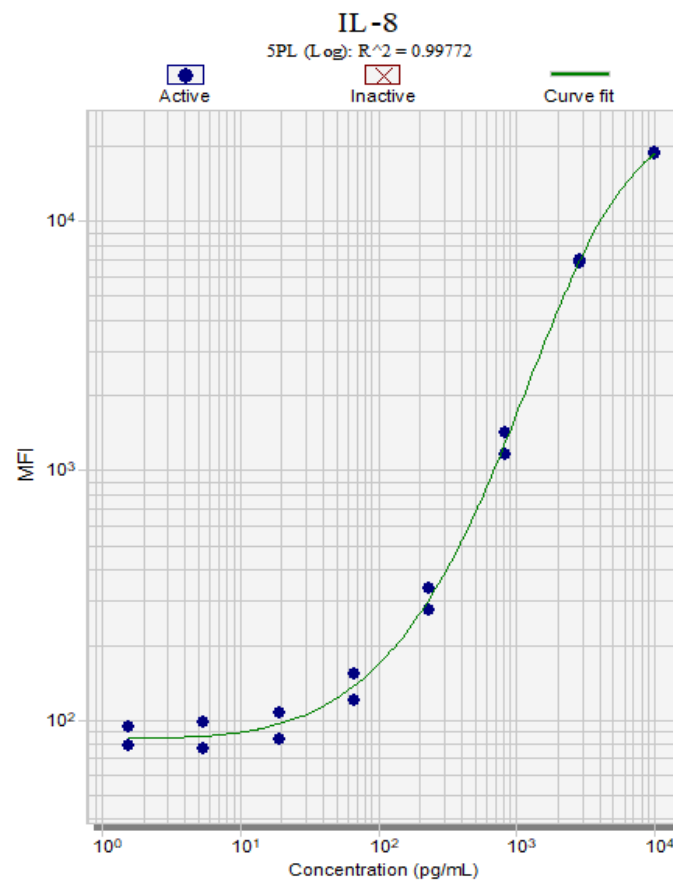
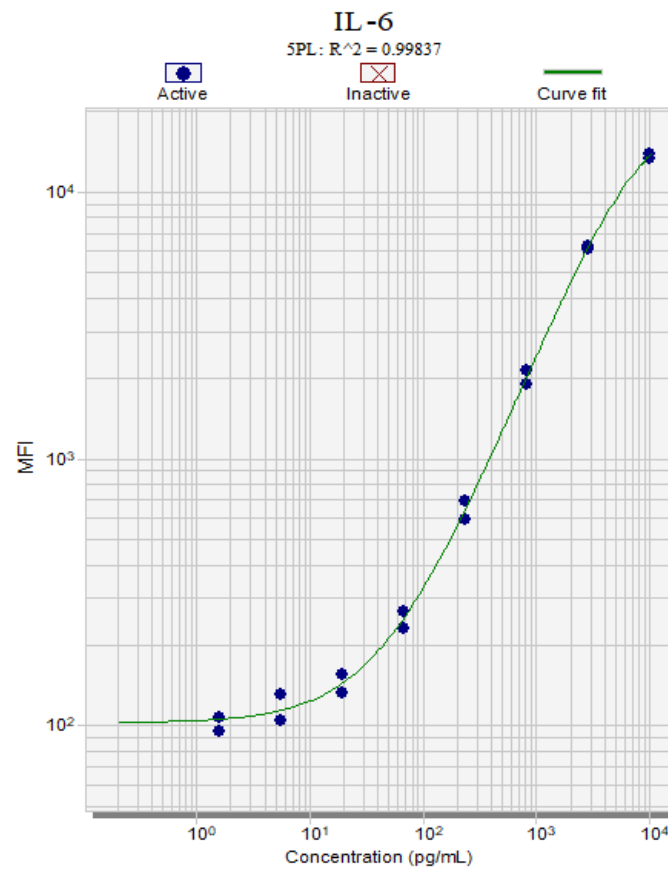
		S1	S8	LDD	LLOQ
IFN γ	pg/mL	1.3	8000	1.3	30
IL-12p40	pg/mL	0.62	4000	1.6	6.6
IL-12p70	pg/mL	1.2	7500	1.2	4.4
IL-17	pg/mL	0.27	1750	2.6	7.9
IL-2	pg/mL	0.47	3000	0.47	2.7
IL-4	pg/mL	0.56	3600	2.8	12
IL-6	pg/mL	1.6	10000	1.8	4.0
IL-8	pg/mL	1.6	10000	1.6	17
IP-10	pg/mL	1.6	10000	1.9	4.6
MCP-1	pg/mL	0.47	3000	0.47	12
MIP-1 β	pg/mL	0.93	6000	6.5	11
TNF α	pg/mL	1.6	10000	1.6	14

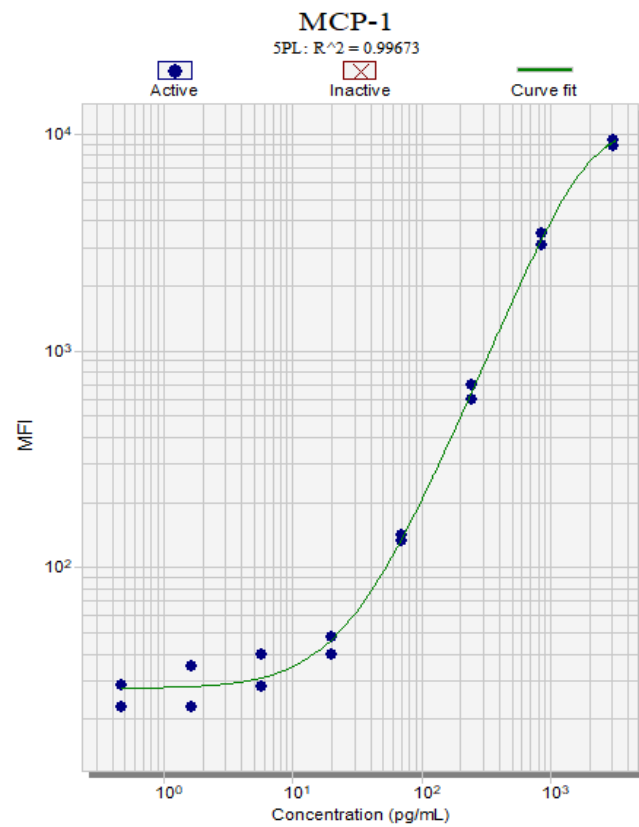
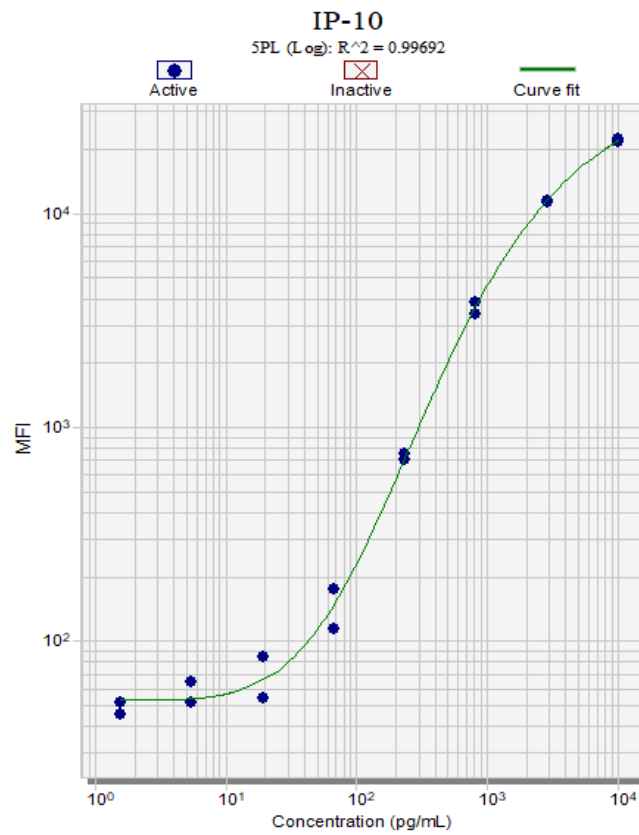
X dilution factor		S1	S8	LDD	LLOQ
IFN γ	pg/mL	3.9	24000	3.9	89
IL-12p40	pg/mL	1.9	12000	4.8	20
IL-12p70	pg/mL	3.6	22500	3.6	13
IL-17	pg/mL	0.81	5250	7.8	24
IL-2	pg/mL	1.4	9000	1.4	8.2
IL-4	pg/mL	1.7	10800	8.4	36
IL-6	pg/mL	4.8	30000	5.4	12
IL-8	pg/mL	4.8	30000	4.8	51
IP-10	pg/mL	4.8	30000	5.7	14
MCP-1	pg/mL	1.4	9000	1.4	36
MIP-1 β	pg/mL	2.8	18000	20	33
TNF α	pg/mL	4.8	30000	4.8	42

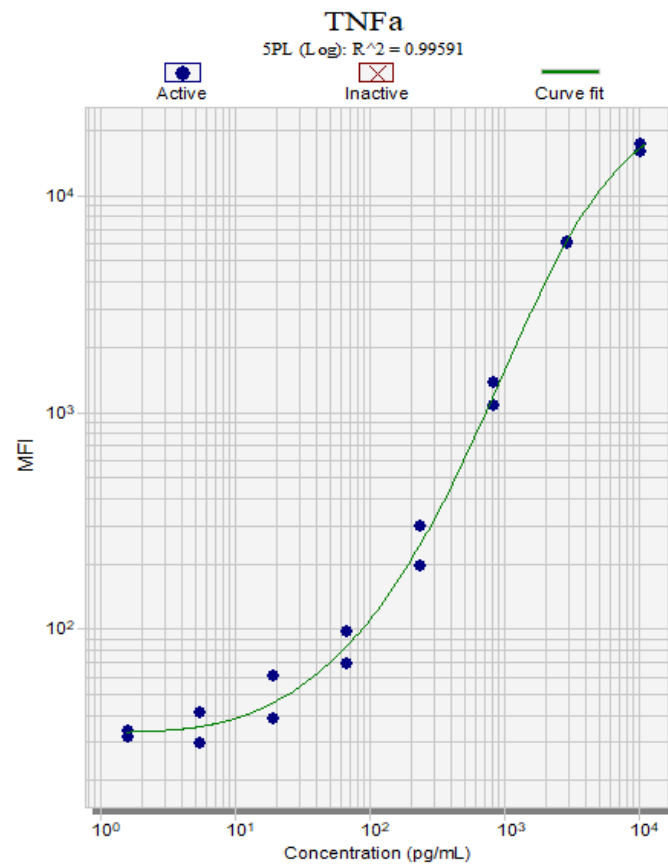
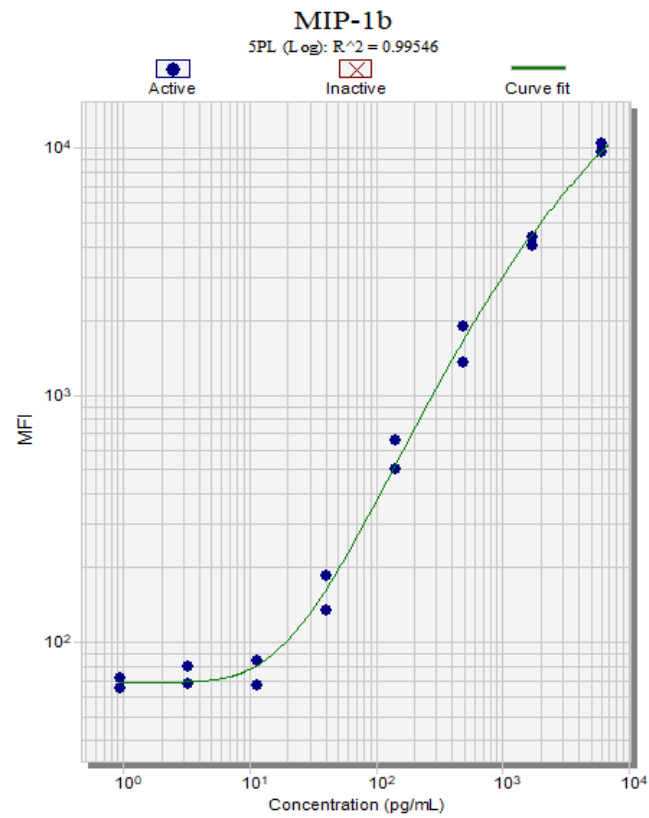












4. Precision:

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.

IFN γ		1	2	3	Inter
Control 1	Mean	470	434	496	467
	% CV	5%	2%	1%	6%
Control 2	Mean	4387	4687	4720	4598
	% CV	1%	0%	6%	5%

IL-12p40		1	2	3	Inter
Control 1	Mean	79	76	88	467
	% CV	3%	2%	5%	7%
Control 2	Mean	531	565	755	617
	% CV	7%	9%	8%	18%

IL-12p70		1	2	3	Inter
Control 1	Mean	253	314	288	285
	% CV	6%	4%	1%	10%
Control 2	Mean	706	844	792	781
	% CV	3%	6%	4%	9%

IL-17		1	2	3	Inter
Control 1	Mean	250	260	206	239
	% CV	1%	1%	1%	10%
Control 2	Mean	405	429	408	414
	% CV	7%	0%	6%	5%

IL-2		1	2	3	Inter
Control 1	Mean	40	38	36	38
	% CV	7%	2%	1%	5%
Control 2	Mean	263	288	338	296
	% CV	2%	1%	9%	12%

IL-4		1	2	3	Inter
Control 1	Mean	722	812	877	804
	% CV	0%	6%	1%	9%
Control 2	Mean	1490	1727	1850	1689
	% CV	7%	4%	8%	11%

IL-6		1	2	3	Inter
Control 1	Mean	87	103	87	93
	% CV	6%	4%	2%	9%
Control 2	Mean	2013	2117	2257	2129
	% CV	3%	2%	4%	6%

IL-8		1	2	3	Inter
Control 1	Mean	812	757	671	747
	% CV	2%	1%	2%	8%
Control 2	Mean	2167	2113	1990	2090
	% CV	6%	4%	5%	6%

IP-10		1	2	3	Inter
Control 1	Mean	159	192	202	184
	% CV	1%	4%	0%	11%
Control 2	Mean	680	750	853	761
	% CV	4%	1%	7%	11%

MCP-1		1	2	3	Inter
Control 1	Mean	226	244	254	241
	% CV	4%	2%	6%	6%
Control 2	Mean	1713	1647	1717	1692
	% CV	5%	4%	8%	5%

MIP-1 β		1	2	3	Inter
Control 1	Mean	72	70	83	75
	% CV	8%	7%	3%	10%
Control 2	Mean	1197	1177	1367	1247
	% CV	7%	3%	8%	9%

TNF α		1	2	3	Inter
Control 1	Mean	71	92	66	76
	% CV	2%	3%	9%	16%
Control 2	Mean	1147	1333	1440	1307
	% CV	3%	2%	4%	10%

5. Linearity:

Linearity was assessed using 2 serum and 2 plasma samples spiked with the standard and diluted 1:2 for 8 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.

IFN γ				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	2705	4205	2985	1260
1:6	1405	2135	1600	574
1:12	697	1165	812	223
1:24	332	629	422	153
2	104%	102%	107%	91%
4	103%	111%	109%	71%
8	98%	120%	113%	97%

IL-12p40				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	346	1055	383	577
1:6	203	588	221	280
1:12	96	309	95	136
1:24	39	167	48	91
2	117%	111%	116%	97%
4	111%	117%	100%	94%
8	90%	127%	101%	126%

IL-12p70				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	178	3730	1080	656
1:6	115	1880	489	341
1:12	50	980	284	210
1:24	23	479	170	105
2	129%	101%	91%	104%
4	112%	105%	105%	128%
8	104%	103%	126%	128%

IL-17				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	558	1475	1485	248
1:6	308	759	821	108
1:12	160	388	376	46
1:24	78	218	199	26
2	110%	103%	111%	87%
4	115%	105%	101%	74%
8	112%	118%	107%	84%

IL-2				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	476	1110	227	45
1:6	225	589	102	25
1:12	107	300	56	13
1:24	53	152	29	5.8
2	95%	106%	90%	110%
4	90%	108%	98%	119%
8	90%	110%	104%	103%

IL-4				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	1320	2180	2155	1110
1:6	617	1120	1205	566
1:12	292	592	615	322
1:24	128	328	315	162
2	93%	103%	112%	102%
4	88%	109%	114%	116%
8	77%	120%	117%	116%

IL-6				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	548	7020	3170	627
1:6	285	3980	1330	351
1:12	127	2135	702	196
1:24	57	1080	337	57
2	104%	113%	84%	112%
4	93%	122%	89%	125%
8	84%	123%	85%	72%

IL-8				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	11267	5820	5160	2115
1:6	5793	3520	2700	1130
1:12	3297	1825	1445	577
1:24	1793	882	705	295
2	103%	121%	105%	107%
4	117%	125%	112%	109%
8	127%	121%	109%	112%

IP-10				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	288	7150	1540	1905
1:6	141	3295	810	994
1:12	63	1740	427	515
1:24	25	983	217	281
2	98%	92%	105%	104%
4	87%	97%	111%	108%
8	70%	110%	113%	118%

MCP-1				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	12833	1130	818	252
1:6	6653	681	366	128
1:12	2947	364	214	76
1:24	1517	172	123	29
2	104%	120%	89%	101%
4	92%	129%	105%	121%
8	95%	122%	120%	93%

MIP-1 β				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:3	538	15800	2315	10040
1:6	273	7740	949	4660
1:12	165	3595	494	2785
1:24	84	1915	236	1490
2	101%	98%	82%	93%
4	123%	91%	85%	111%
8	125%	97%	81%	119%

TNF α					
pg/mL		Serum 1	Serum 2	Plasma 1	Plasma 2
1:3		1225	12550	2330	545
1:6		622	6835	1085	238
1:12		247	3570	479	135
1:24		113	1805	252	51
2		102%	109%	93%	87%
4		81%	114%	82%	99%
8		74%	115%	86%	75%

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.

IFN γ					
pg/mL		Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	2990	1385	2530	3750
	FT-1X	3005	1480	2410	3720
	FT-2X	3165	1465	2440	3780
	FT-3X	3085	1490	2555	3860
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	101%	107%	95%	99%
	FT-2X	106%	106%	96%	101%
	FT-3X	103%	108%	101%	103%

IL-12p40					
pg/mL		Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	370	518	534	1770
	FT-1X	391	547	499	1700
	FT-2X	418	555	582	1725
	FT-3X	405	583	545	1740
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	106%	106%	93%	96%
	FT-2X	113%	107%	109%	97%
	FT-3X	109%	113%	102%	98%

	IL-12p70				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	986	611	472	1780
	FT-1X	1083	616	411	1820
	FT-2X	1054	559	476	1840
	FT-3X	1100	609	416	1875
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	110%	101%	87%	102%
	FT-2X	107%	91%	101%	103%
	FT-3X	112%	100%	88%	105%

	IL-17				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	1830	245	207	2100
	FT-1X	1830	237	203	2045
	FT-2X	1990	249	207	2010
	FT-3X	2020	249	191	2155
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	100%	97%	98%	97%
	FT-2X	109%	101%	100%	96%
	FT-3X	110%	101%	92%	103%

	IL-2				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	195	55	873	336
	FT-1X	188	54	794	323
	FT-2X	218	57	793	341
	FT-3X	206	60	789	324
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	96%	98%	91%	96%
	FT-2X	112%	104%	91%	101%
	FT-3X	106%	108%	90%	96%

	IL-4				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	1905	1140	909	1995
	FT-1X	1895	1085	959	2005
	FT-2X	1920	1105	978	1990
	FT-3X	1925	1130	962	2085
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	99%	95%	106%	101%
	FT-2X	101%	97%	108%	100%
	FT-3X	101%	99%	106%	105%

	IL-6				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	2895	629	775	7295
	FT-1X	2865	611	700	7215
	FT-2X	2875	571	634	6915
	FT-3X	2900	602	654	7265
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	99%	97%	90%	99%
	FT-2X	99%	91%	82%	95%
	FT-3X	100%	96%	84%	100%

	IL-8				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	4335	2075	1670	6010
	FT-1X	4365	2120	1615	6015
	FT-2X	4665	2100	1585	5960
	FT-3X	4650	2010	1565	6095
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	101%	102%	97%	100%
	FT-2X	108%	101%	95%	99%
	FT-3X	107%	97%	94%	101%

	IP-10				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	1215	1460	4645	2225
	FT-1X	1225	1365	4245	2175
	FT-2X	1325	1455	4260	2160
	FT-3X	1280	1420	4455	2180
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	101%	93%	91%	98%
	FT-2X	109%	100%	92%	97%
	FT-3X	105%	97%	96%	98%

	MCP-1				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	855	724	311	1370
	FT-1X	825	724	289	1200
	FT-2X	851	743	294	1305
	FT-3X	827	730	273	1320
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	96%	100%	93%	88%
	FT-2X	100%	103%	95%	95%
	FT-3X	97%	101%	88%	96%

	MIP-1 β				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	1780	11350	659	2480
	FT-1X	1830	11550	662	2415
	FT-2X	2165	11900	711	2585
	FT-3X	2175	12100	704	2755
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	103%	102%	100%	97%
	FT-2X	122%	105%	108%	104%
	FT-3X	122%	107%	107%	111%

	TNF α				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	FT-0X	2275	624	8420	4665
	FT-1X	2360	638	7735	4540
	FT-2X	2385	612	7545	4505
	FT-3X	2420	622	7615	4485
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	104%	102%	92%	97%
	FT-2X	105%	98%	90%	97%
	FT-3X	106%	100%	90%	96%

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.

	IFN γ				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	3155	1185	2375	3655
	2hr RT	2650	1185	2260	3590
	2hr 4C	2680	1310	2100	3575
	4hr 4C	2845	1285	2430	3775
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	84%	100%	95%	98%
	2hr 4C	85%	111%	88%	98%
	4hr 4C	90%	108%	102%	103%

	IL-12p40				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	380	404	448	1465
	2hr RT	321	422	408	1470
	2hr 4C	351	431	358	1555
	4hr 4C	338	442	462	1590
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	84%	104%	91%	100%
	2hr 4C	92%	107%	80%	106%
	4hr 4C	89%	109%	103%	109%

	IL-12p70				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	1093	575	455	1960
	2hr RT	924	559	439	2095
	2hr 4C	968	573	384	1840
	4hr 4C	947	582	454	1960
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	84%	97%	96%	107%
	2hr 4C	89%	100%	84%	94%
	4hr 4C	87%	101%	100%	100%

	IL-17				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	2155	219	178	2125
	2hr RT	1780	224	177	2130
	2hr 4C	1760	225	172	2085
	4hr 4C	1850	235	190	2180
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	83%	103%	100%	100%
	2hr 4C	82%	103%	97%	98%
	4hr 4C	86%	107%	107%	103%

	IL-2				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	203	39	797	313
	2hr RT	161	41	795	296
	2hr 4C	160	42	754	292
	4hr 4C	171	39	823	311
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	80%	105%	100%	95%
	2hr 4C	79%	107%	95%	93%
	4hr 4C	84%	101%	103%	100%

	IL-4				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	2180	1049	774	2080
	2hr RT	1825	1019	932	2060
	2hr 4C	1845	1075	989	1965
	4hr 4C	1885	1070	914	2055
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	84%	97%	120%	99%
	2hr 4C	85%	102%	128%	94%
	4hr 4C	86%	102%	118%	99%

	IL-6				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	3210	577	708	7515
	2hr RT	2650	558	535	7270
	2hr 4C	2530	564	541	7080
	4hr 4C	2635	589	547	7290
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	83%	97%	76%	97%
	2hr 4C	79%	98%	76%	94%
	4hr 4C	82%	102%	77%	97%

	IL-8				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	5290	1950	1420	6015
	2hr RT	4500	1990	1495	5990
	2hr 4C	4310	2030	1525	5895
	4hr 4C	4485	2055	1575	6150
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	85%	102%	105%	100%
	2hr 4C	81%	104%	107%	98%
	4hr 4C	85%	105%	111%	102%

	IP-10				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	1390	1360	4365	2185
	2hr RT	1095	1260	4115	2080
	2hr 4C	1250	1380	4150	2120
	4hr 4C	1155	1385	4535	2215
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	79%	93%	94%	95%
	2hr 4C	90%	101%	95%	97%
	4hr 4C	83%	102%	104%	101%

	MCP-1				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	1014	740	235	1360
	2hr RT	835	753	270	1240
	2hr 4C	831	740	258	1290
	4hr 4C	912	744	296	1325
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	82%	102%	115%	91%
	2hr 4C	82%	100%	110%	95%
	4hr 4C	90%	101%	126%	97%

	MIP-1β				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	1610	8915	488	1890
	2hr RT	1330	8515	435	1870
	2hr 4C	1355	9810	484	2045
	4hr 4C	1565	10800	568	2260
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	83%	96%	89%	99%
	2hr 4C	84%	110%	99%	108%
	4hr 4C	97%	121%	117%	120%

	TNFα				
	pg/mL	Plasma 1	Plasma 2	Serum 1	Serum 2
Value	CTL-0 Hr	2790	551	8560	5105
	2hr RT	2250	496	7880	4715
	2hr 4C	2215	535	7650	4720
	4hr 4C	2405	547	8340	4855
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	81%	90%	92%	92%
	2hr 4C	79%	97%	89%	92%
	4hr 4C	86%	99%	97%	95%