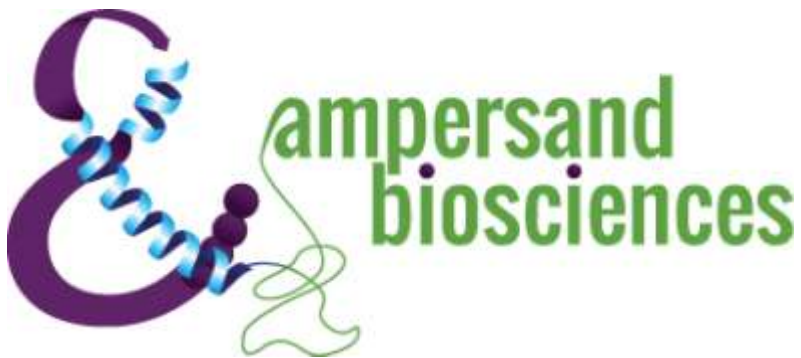


Mouse Cytokine-Chemokine Panel 2

Kit # M105-K

Validation Report Version 1.0

May 24, 2024



1. Assay Description:

A multiplex assay was developed and validated for the measurement of Mouse CCL1, CCL6, Eotaxin, GCP-2, IP-10, ITAC, MCP-1, M-CSF, MDC, MIP-1 α , and MIP-1 β . 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 (0.625%) spiked with Recombinant CCL1, CCL6, Eotaxin, GCP-2, IP-10, ITAC, MCP-1, MDC, MIP-1 α and MIP-1 β .
Control 2	Normal Mouse Serum (20%) spiked with Recombinant CCL1, CCL6, Eotaxin, GCP-2, IP-10, ITAC, MCP-1, MDC, MIP-1 α and MIP-1 β .

Sample	Description
Serum 1	Normal Mouse Serum (10%) spiked with Recombinant CCL1, CCL6, Eotaxin, GCP-2, IP-10, ITAC, MCP-1, MDC, MIP-1 α and MIP-1 β .
Serum 2	Normal Mouse Serum (99%) spiked with Recombinant ITAC, MCP-1 and MIP-1 α .
Plasma 1	Normal Mouse Plasma (99%) spiked with Recombinant GCP-2, ITAC, MCP-1 and MIP-1 α .
Plasma 2	Normal Mouse Plasma (99%) spiked with Recombinant GCP-2, ITAC, MCP-1 and MIP-1 α .

3. LLOQ, LDD and Curves:

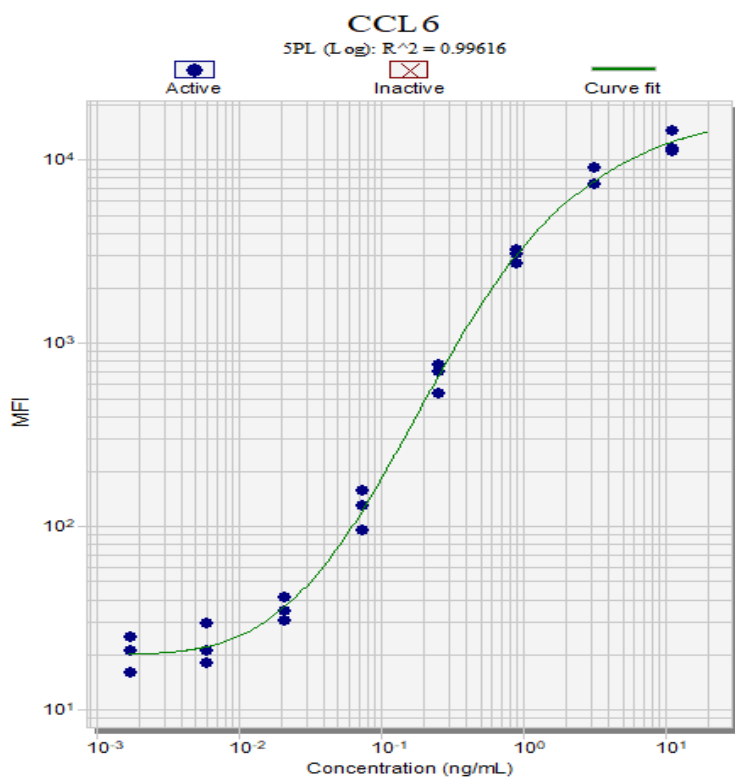
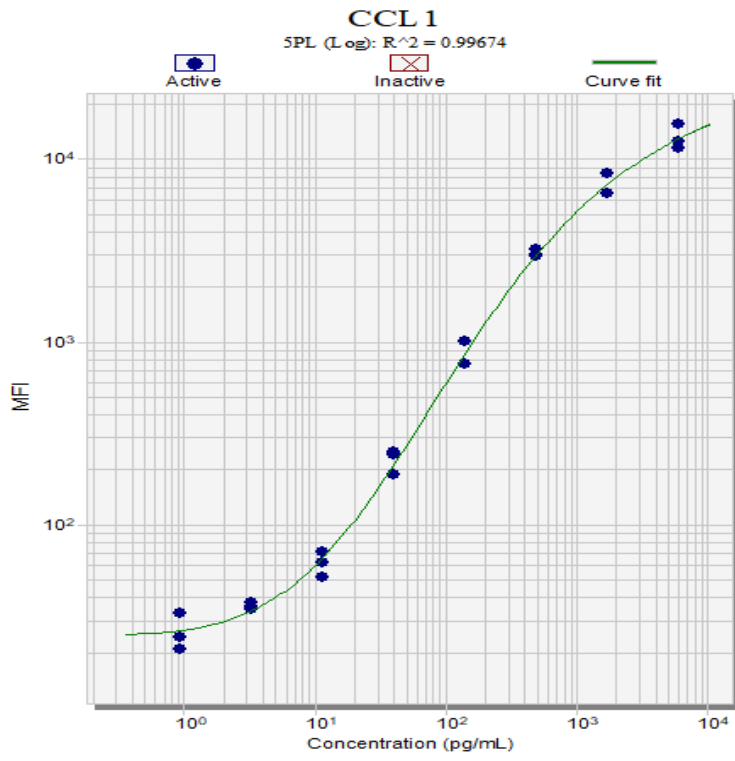
LDD: MFI (Median Fluorescent Intensity) for 20 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

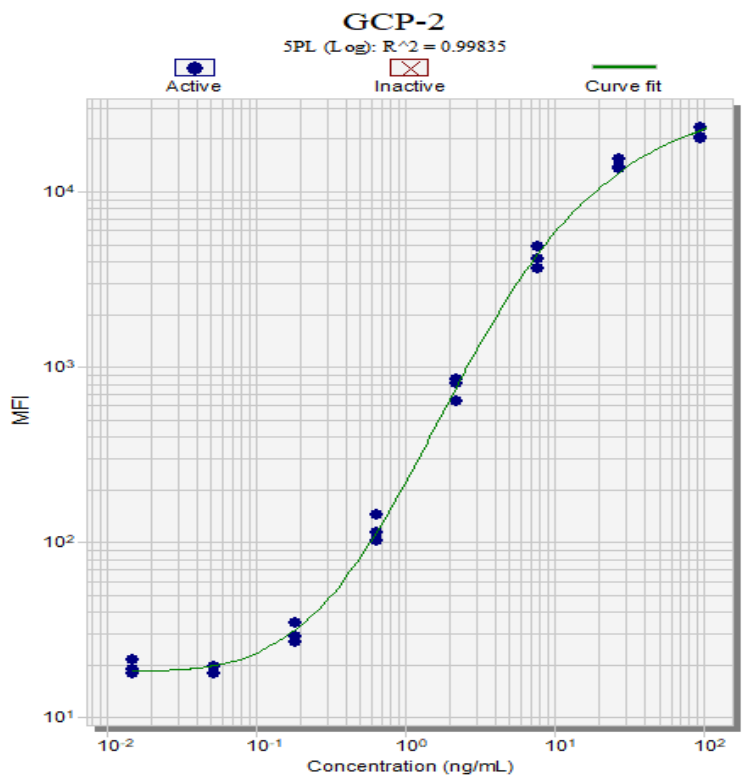
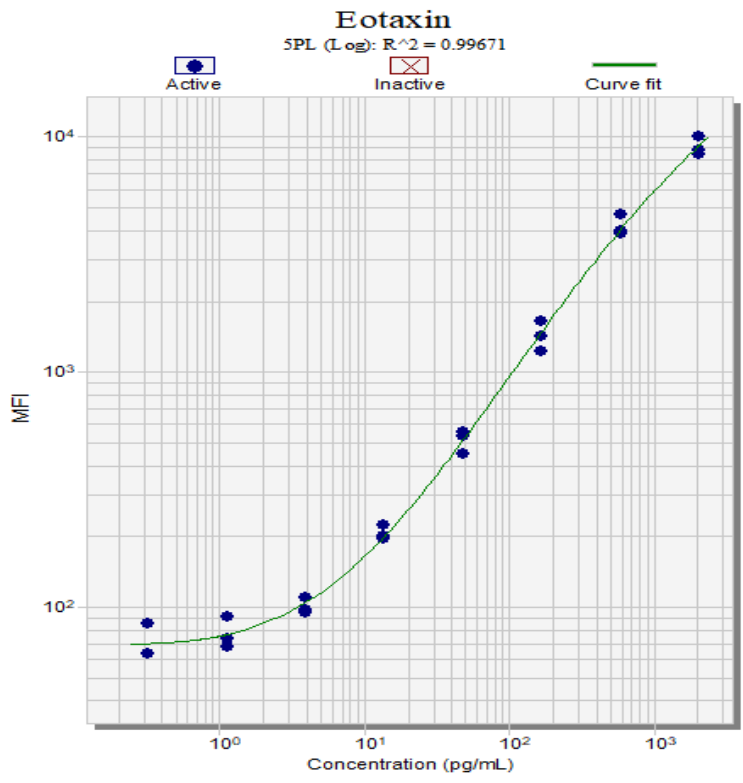
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in triplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

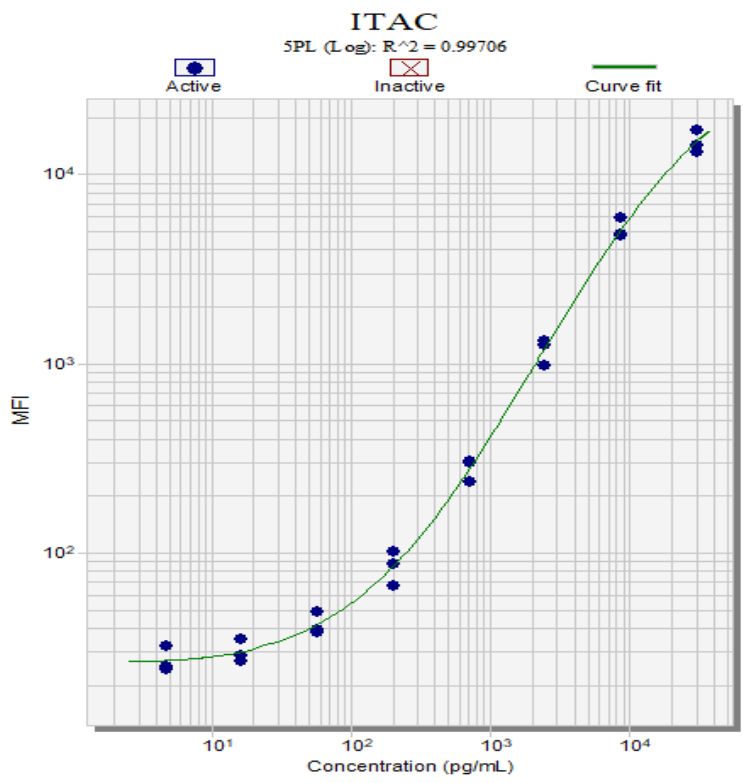
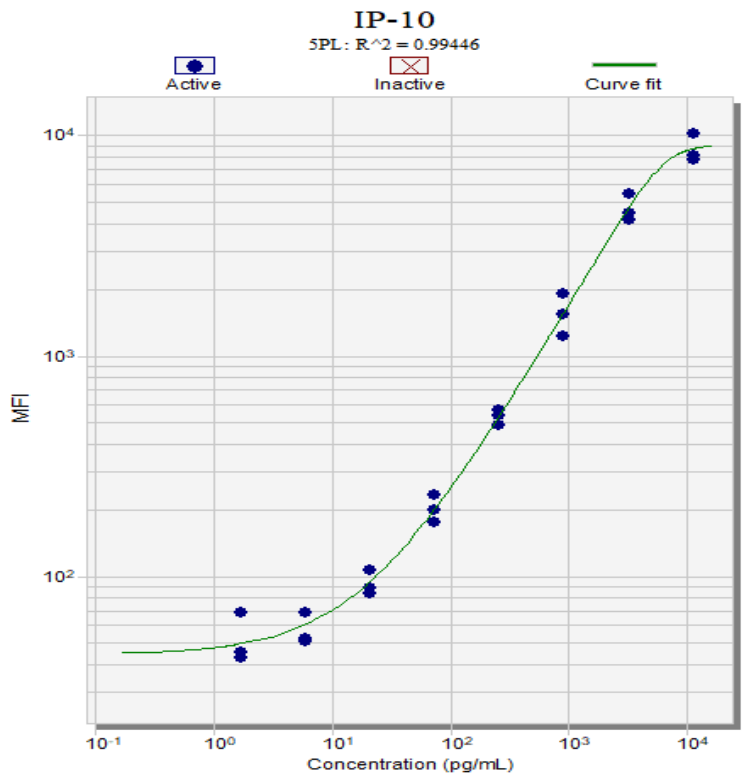
Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.

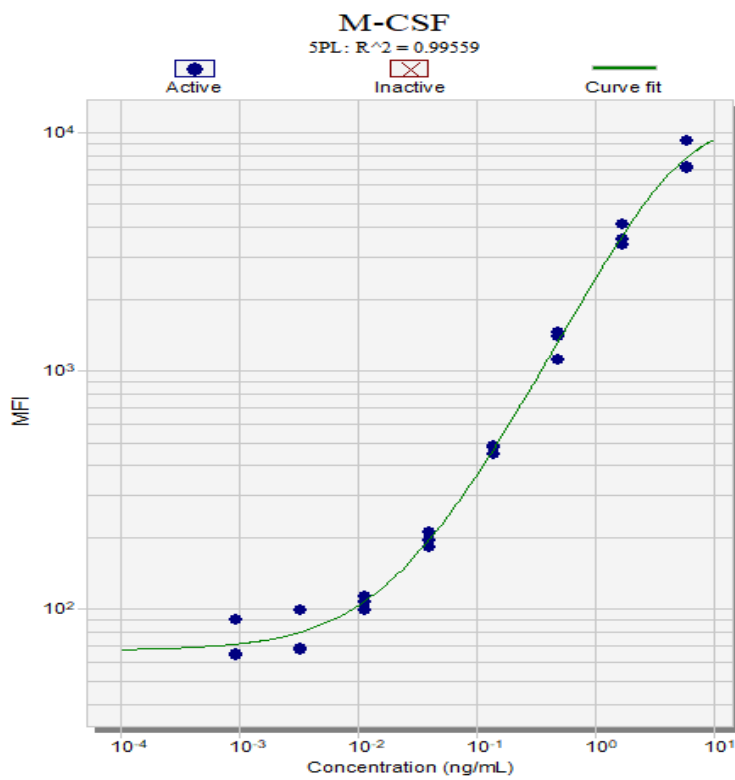
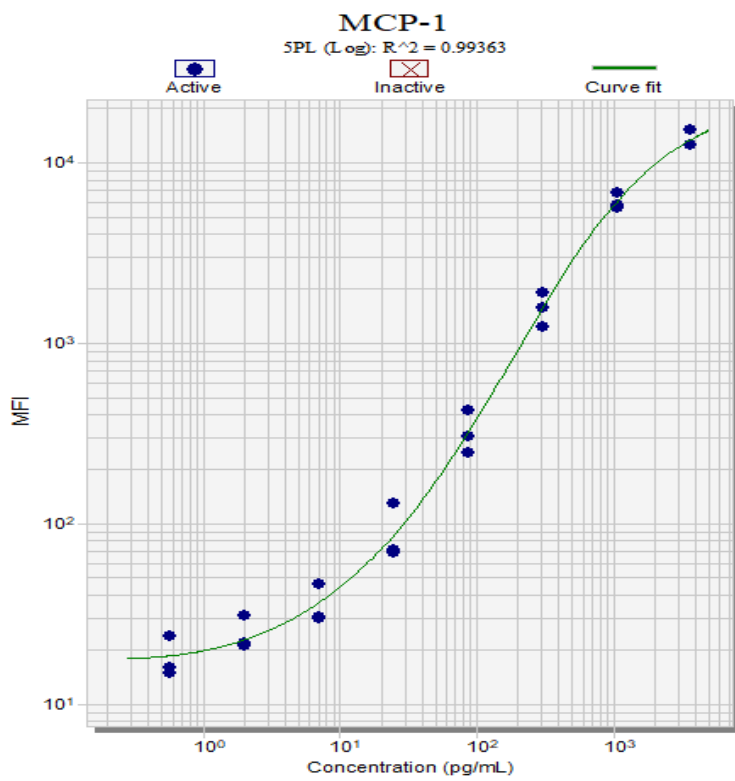
		S1	S8	LDD	LLOQ
CCL1	pg/mL	0.931	6000	1.2	4.1
CCL6	ng/mL	0.00171	11	0.0110	0.017
Eotaxin	pg/mL	0.317	2045	0.71	2.9
GCP-2	ng/mL	0.0147	95	0.058	0.090
IP-10	pg/mL	1.71	11000	3.3	11
ITAC	pg/mL	4.66	30000	15	28
MCP-1	pg/mL	0.571	3680	1.8	3.6
M-CSF	ng/mL	0.000931	6	0.0017	0.0093
MDC	pg/mL	0.207	1340	0.29	0.81
MIP-1α	pg/mL	47.4	304500	367	449
MIP-1β	pg/mL	0.351	2250	0.40	1.5

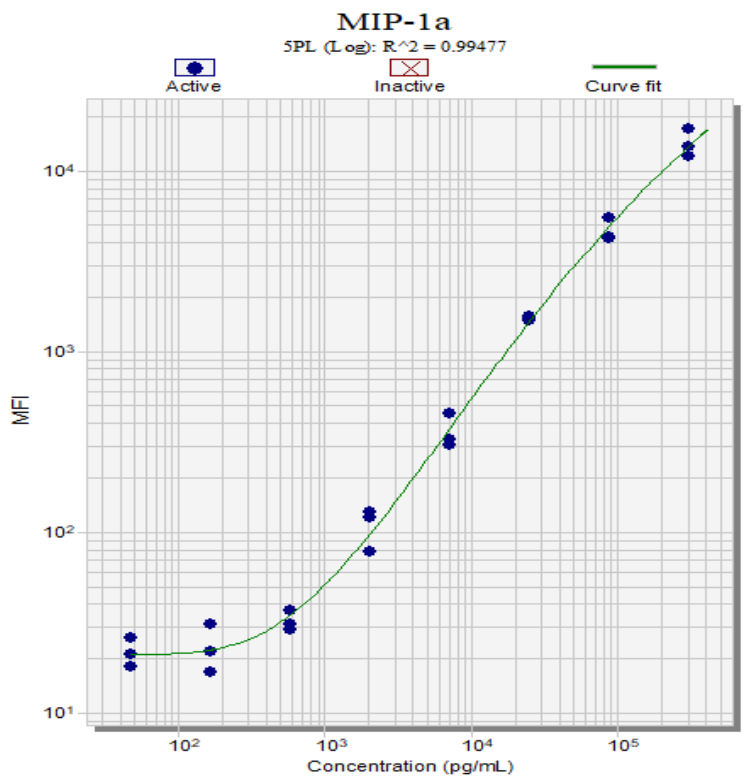
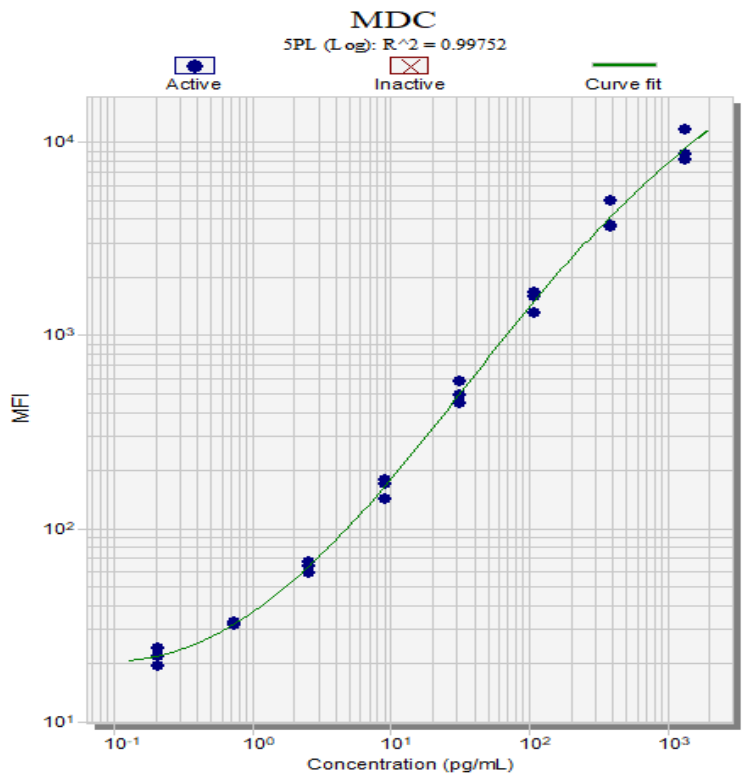
X Dilution Factor		S1	S8	LDD	LLOQ
CCL1	pg/mL	4.655	30000	6.0	21
CCL6	ng/mL	0.00855	55	0.055	0.087
Eotaxin	pg/mL	1.585	10225	3.6	15
GCP-2	ng/mL	0.0735	475	0.29	0.45
IP-10	pg/mL	8.55	55000	17	54
ITAC	pg/mL	23.3	150000	74	139
MCP-1	pg/mL	2.855	18400	9.0	18
M-CSF	ng/mL	0.004655	30	0.0085	0.046
MDC	pg/mL	1.035	6700	1.5	4.1
MIP-1α	pg/mL	237	1522500	1833	2244
MIP-1β	pg/mL	1.755	11250	2.0	7.6

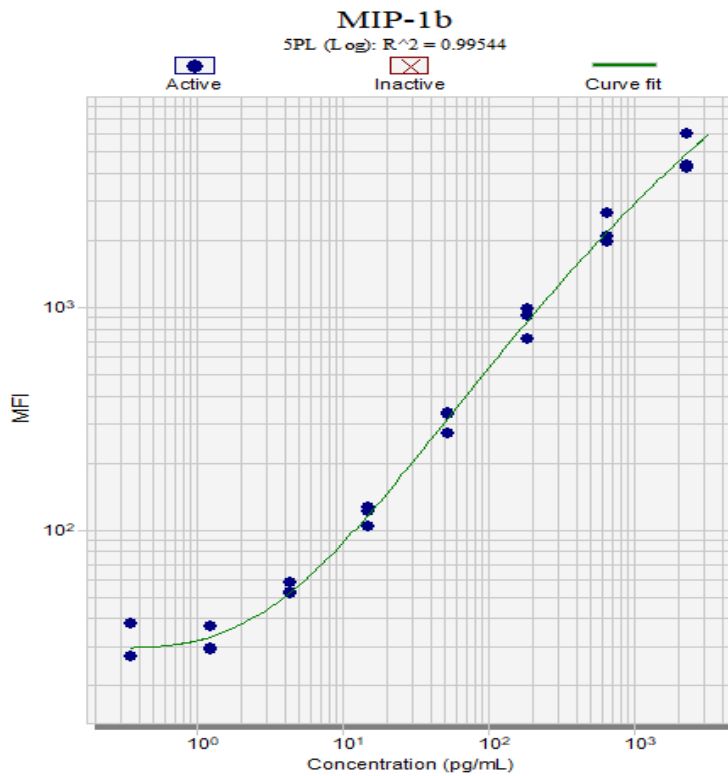












4. Precision:

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.

CCL1		1	2	3	Inter
Control 1	Mean	16	13	18	16
	% CV	10%	1%	3%	13%
Control 2	Mean	758	789	757	768
	% CV	3%	3%	13%	4%
CCL6		1	2	3	Inter
Control 1	Mean	0.057	0.045	0.060	0.054
	% CV	9%	15%	1%	15%
Control 2	Mean	2.4	1.7	2.3	2.1
	% CV	3%	1%	4%	15%

Eotaxin		1	2	3	Inter
Control 1	Mean	7.7	7.4	10	8.4
	% CV	17%	6%	1%	17%
Control 2	Mean	388	362	421	390
	% CV	1%	3%	7%	8%

GCP-2		1	2	3	Inter
Control 1	Mean	0.46	0.39	0.37	0.41
	% CV	9%	9%	9%	13%
Control 2	Mean	8.6	6.8	8.7	8.0
	% CV	1%	3%	2%	12%

IP-10		1	2	3	Inter
Control 1	Mean	24	33	34	31
	% CV	4%	8%	7%	15%
Control 2	Mean	1380	1233	1633	1416
	% CV	1%	1%	5%	13%

ITAC		1	2	3	Inter
Control 1	Mean	204	148	202	185
	% CV	8%	2%	4%	16%
Control 2	Mean	2990	2957	3883	3277
	% CV	3%	1%	3%	14%

MCP-1		1	2	3	Inter
Control 1	Mean	16	12	17	15
	% CV	11%	10%	1%	16%
Control 2	Mean	759	641	733	711
	% CV	2%	5%	2%	8%

M-CSF		1	2	3	Inter
Control 1	Mean	0.075	0.12	0.094	0.10
	% CV	4%	4%	2%	19%
Control 2	Mean	1.1	1.0	1.3	1.1
	% CV	4%	2%	3%	13%

MDC		1	2	3	Inter
Control 1	Mean	2.1	1.8	2.4	2.1
	% CV	11%	11%	4%	14%
Control 2	Mean	118	107	122	116
	% CV	6%	5%	10%	9%

MIP-1 α		1	2	3	Inter
Control 1	Mean	2993	2403	2737	2711
	% CV	7%	4%	3%	10%
Control 2	Mean	56767	51400	72400	60189
	% CV	4%	1%	2%	16%

MIP-1 β		1	2	3	Inter
Control 1	Mean	21	19	21	20
	% CV	13%	6%	3%	10%
Control 2	Mean	767	919	956	880
	% CV	9%	12%	7%	13%

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.

CCL1				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	1670	143	190	243
1:4	673	74	105	148
1:8	302	43	60	70
1:16	172	27	35	40
2	81%	103%	110%	122%
4	72%	117%	115%	95%
8	82%	126%	116%	113%

CCL6				
ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	6.5	9.2	15	18
1:4	4.0	3.8	5.9	12
1:8	2.1	1.8	3.3	7.1
1:16	1.2	1.0	2.0	3.8
2	124%	83%	77%	130%
4	104%	81%	86%	122%
8	119%	87%	105%	107%

Eotaxin				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	1435	2355	3190	3290
1:4	813	1255	1625	1730
1:8	392	608	848	903
1:16	205	343	431	419
2	113%	107%	102%	105%
4	96%	97%	104%	104%
8	105%	113%	102%	93%

GCP-2				
ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	26	13	35	85
1:4	11	7.5	17	52
1:8	5.1	4.0	8.8	29
1:16	2.4	2.1	5.0	17
2	84%	113%	96%	121%
4	78%	120%	101%	112%
8	73%	125%	114%	120%

IP-10				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	3125	5187	605	668
1:4	1860	2403	282	334
1:8	992	1096	103	119
1:16	563	571	<LLOQ	<LLOQ
2	119%	93%	93%	100%
4	107%	84%	73%	71%
8	114%	88%	<LLOQ	<LLOQ

ITAC				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	10010	34100	13000	19700
1:4	5070	19750	5770	10020
1:8	2245	7850	3385	5875
1:16	1110	4445	2070	2945
2	101%	116%	89%	102%
4	89%	79%	117%	117%
8	99%	113%	122%	100%

MCP-1				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	1383	2805	2015	3200
1:4	578	1410	924	1940
1:8	253	665	463	1060
1:16	133	371	231	615
2	84%	101%	92%	121%
4	73%	95%	92%	109%
8	77%	106%	92%	116%

M-CSF				
ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	2.9	5.8	2.8	4.2
1:4	1.2	2.8	1.4	2.6
1:8	0.56	1.2	0.73	1.5
1:16	0.36	0.62	0.42	0.84
2	85%	98%	97%	123%
4	76%	84%	103%	118%
8	98%	86%	118%	110%

MDC				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	438	493	321	379
1:4	263	289	168	218
1:8	129	139	93	118
1:16	74	80	49	57
2	120%	117%	104%	115%
4	98%	96%	110%	108%
8	114%	116%	105%	96%

MIP-1α				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	85800	140500	92900	137000
1:4	43050	78100	47350	73000
1:8	18300	37000	22450	36700
1:16	8175	18400	11150	16450
2	100%	111%	102%	107%
4	85%	95%	95%	101%
8	89%	99%	99%	90%

MIP-1β				
pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
1:2	1135	134	125	149
1:4	689	76	68	83
1:8	370	47	32	42
1:16	222	22	14	16
2	121%	113%	110%	111%
4	107%	125%	95%	101%
8	120%	95%	89%	74%

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.

	CCL1				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	264	70	110	137
	FT-1X	273	77	97	129
	FT-2X	254	79	107	164
	FT-3X	245	68	109	129
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	103%	109%	88%	95%
	FT-2X	96%	112%	97%	120%
	FT-3X	93%	97%	99%	95%

	CCL6				
	ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	1.0	0.26	15	15
	FT-1X	1.1	0.26	13	14
	FT-2X	1.0	0.26	13	14
	FT-3X	1.0	0.23	16	14
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	101%	99%	89%	93%
	FT-2X	100%	99%	90%	94%
	FT-3X	98%	87%	106%	91%

	Eotaxin				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	119	1280	2055	2100
	FT-1X	109	1245	1840	1930
	FT-2X	116	1285	2010	1900
	FT-3X	117	1245	2225	1970
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	92%	97%	90%	92%
	FT-2X	98%	100%	98%	90%
	FT-3X	98%	97%	108%	94%

		GCP-2				
		ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		3.3	14	36	55
	FT-1X		3.3	14	33	52
	FT-2X		3.0	14	35	65
	FT-3X		2.9	14	35	53
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		101%	99%	91%	95%
	FT-2X		93%	104%	96%	119%
	FT-3X		90%	100%	97%	97%

		IP-10				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		372	71	403	439
	FT-1X		374	52	352	394
	FT-2X		390	67	381	384
	FT-3X		334	60	435	402
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		101%	73%	87%	90%
	FT-2X		105%	94%	95%	87%
	FT-3X		90%	85%	108%	92%

		ITAC				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		497	21100	8300	11800
	FT-1X		522	20250	6290	10600
	FT-2X		527	21000	6495	12700
	FT-3X		431	19950	7085	8890
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		105%	96%	76%	90%
	FT-2X		106%	100%	78%	108%
	FT-3X		87%	95%	85%	75%

		MCP-1				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		150	2150	2025	2760
	FT-1X		159	2115	1820	2630
	FT-2X		152	2210	1930	3320
	FT-3X		157	2090	2105	2660
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		106%	98%	90%	95%
	FT-2X		101%	103%	95%	120%
	FT-3X		105%	97%	104%	96%

	M-CSF				
	ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	0.38	3.9	2.8	3.3
	FT-1X	0.40	3.9	2.7	3.3
	FT-2X	0.40	3.9	2.9	3.5
	FT-3X	0.41	3.8	3.0	3.3
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	103%	100%	95%	98%
	FT-2X	104%	101%	102%	105%
	FT-3X	106%	98%	104%	98%

	MDC				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	50	282	193	220
	FT-1X	52	277	177	208
	FT-2X	49	283	187	212
	FT-3X	50	267	208	215
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	103%	98%	91%	94%
	FT-2X	98%	100%	97%	96%
	FT-3X	99%	95%	108%	98%

	MIP-1 α				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	8025	114500	75900	121000
	FT-1X	7800	108000	69500	111000
	FT-2X	7500	116000	73050	128000
	FT-3X	8095	107500	81150	111500
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	97%	94%	92%	92%
	FT-2X	93%	101%	96%	106%
	FT-3X	101%	94%	107%	92%

	MIP-1 β				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	198	81	80	97
	FT-1X	192	79	76	90
	FT-2X	202	82	75	109
	FT-3X	189	75	87	97
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	97%	97%	95%	94%
	FT-2X	102%	100%	93%	113%
	FT-3X	95%	93%	108%	100%

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.

		CCL1				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr		283	99	131	141
	2hr RT		300	69	129	144
	2hr 4C		267	82	120	125
	4hr 4C		293	84	120	134
% Control	CTL-0 Hr		100%	100%	100%	100%
	2hr RT		106%	70%	98%	102%
	2hr 4C		95%	83%	91%	88%
	4hr 4C		104%	86%	91%	95%

		CCL6				
		ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr		1.1	0.33	17	15
	2hr RT		0.94	0.28	14	14
	2hr 4C		1.1	0.30	14	13
	4hr 4C		1.1	0.29	14	14
% Control	CTL-0 Hr		100%	100%	100%	100%
	2hr RT		86%	86%	83%	95%
	2hr 4C		97%	91%	84%	85%
	4hr 4C		96%	88%	83%	89%

		Eotaxin				
		pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr		117	1590	2225	2070
	2hr RT		138	1120	2140	1995
	2hr 4C		123	1235	1980	1745
	4hr 4C		135	1355	2115	2025
% Control	CTL-0 Hr		100%	100%	100%	100%
	2hr RT		118%	70%	96%	96%
	2hr 4C		105%	78%	89%	84%
	4hr 4C		115%	85%	95%	98%

		GCP-2			
		ng/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	3.5	17	40	55
	2hr RT	4.0	14	36	55
	2hr 4C	3.6	15	37	50
	4hr 4C	3.5	16	36	52
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	114%	81%	91%	100%
	2hr 4C	104%	88%	92%	91%
	4hr 4C	99%	91%	91%	95%

		IP-10			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	386	221	501	488
	2hr RT	364	259	429	425
	2hr 4C	385	224	423	381
	4hr 4C	384	257	448	425
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	94%	100%	86%	87%
	2hr 4C	100%	117%	85%	78%
	4hr 4C	99%	101%	90%	87%

		ITAC			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	665	8415	12250	11705
	2hr RT	685	10285	8580	14065
	2hr 4C	654	8050	12650	12595
	4hr 4C	659	9360	12500	14540
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	103%	122%	70%	120%
	2hr 4C	98%	96%	103%	108%
	4hr 4C	99%	111%	102%	124%

		MCP-1			
		pg/mL			
		Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	206	2610	2250	2850
	2hr RT	249	1880	2000	2770
	2hr 4C	206	2100	1930	2480
	4hr 4C	200	2185	2010	2700
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	121%	72%	89%	97%
	2hr 4C	100%	80%	86%	87%
	4hr 4C	97%	84%	89%	95%

	M-CSF				
	ng/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	0.45	5.0	3.2	3.5
	2hr RT	0.49	3.5	3.1	3.5
	2hr 4C	0.46	3.8	2.9	3.2
	4hr 4C	0.46	4.2	3.1	3.3
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	108%	71%	95%	98%
	2hr 4C	101%	77%	90%	90%
	4hr 4C	101%	85%	95%	92%

	MDC				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	53	339	211	221
	2hr RT	58	247	204	220
	2hr 4C	52	266	187	194
	4hr 4C	56	309	198	224
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	109%	73%	97%	100%
	2hr 4C	99%	79%	89%	88%
	4hr 4C	107%	91%	94%	101%

	MIP-1α				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	10260	140000	86900	121500
	2hr RT	10160	100950	77400	119000
	2hr 4C	9900	112500	76450	105500
	4hr 4C	9025	118500	79700	111500
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	99%	72%	89%	98%
	2hr 4C	96%	80%	88%	87%
	4hr 4C	88%	85%	92%	92%

	MIP-1β				
	pg/mL	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	CTL-0 Hr	204	99	96	104
	2hr RT	212	74	87	97
	2hr 4C	209	82	85	88
	4hr 4C	216	89	85	97
% Control	CTL-0 Hr	100%	100%	100%	100%
	2hr RT	104%	74%	91%	94%
	2hr 4C	102%	83%	88%	84%
	4hr 4C	106%	89%	88%	93%