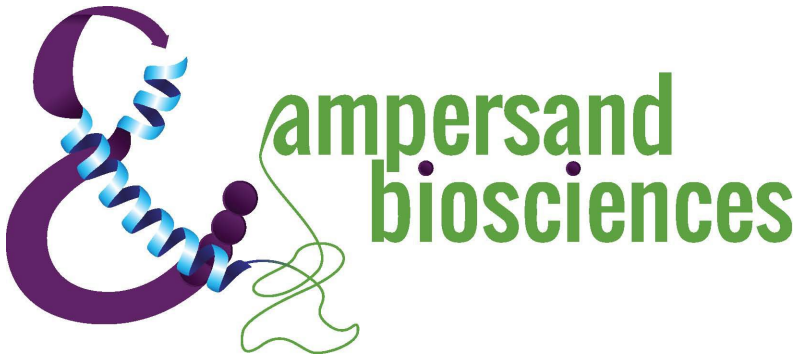


Guinea Pig Panel 1

Kit # G123-K

Validation Report Version 1.0



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Reviewed by: *Laurie Stephen*

Date 04/01/2026

1. Assay Description:

A multiplex assay was developed and validated for the measurement of Guinea Pig IL-1 β , IL-6, IL-8, MIP-1 β and TNF α . The kit is microsphere-based and consists 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 10 minutes, the plate is then analyzed on the Luminex 200 Analyzer.

2. Control and Sample Description:

Control	Description
Control 1	Normal Guinea Pig Serum (20%) spiked with low levels of Recombinant IL-1 β , IL-6, IL-8, MIP-1 β and TNF α .
Control 2	Normal Guinea Pig Serum (20%) spiked with low levels of Recombinant IL-1 β , IL-6, IL-8, MIP-1 β and TNF α .

Sample	Description
Serum 1	Normal Guinea Pig Serum spiked with Recombinant IL-1 β , IL-6, IL-8, MIP-1 β and TNF α .
Serum 2	Normal Guinea Pig Serum spiked with Recombinant IL-1 β , IL-6, IL-8, MIP-1 β and TNF α .
Plasma 1	Normal Guinea Pig Plasma spiked with Recombinant IL-1 β , IL-6, IL-8, MIP-1 β and TNF α .
Plasma 2	Normal Guinea Pig Plasma spiked with Recombinant IL-1 β , IL-6, IL-8, MIP-1 β and TNF α .

3. LLOQ, LDD and Curves:

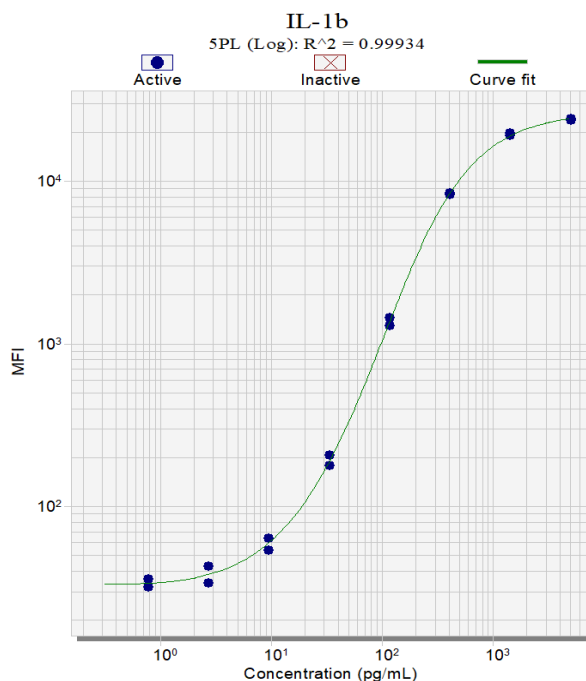
LDD: MFI (Median Fluorescent Intensity) for 8 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

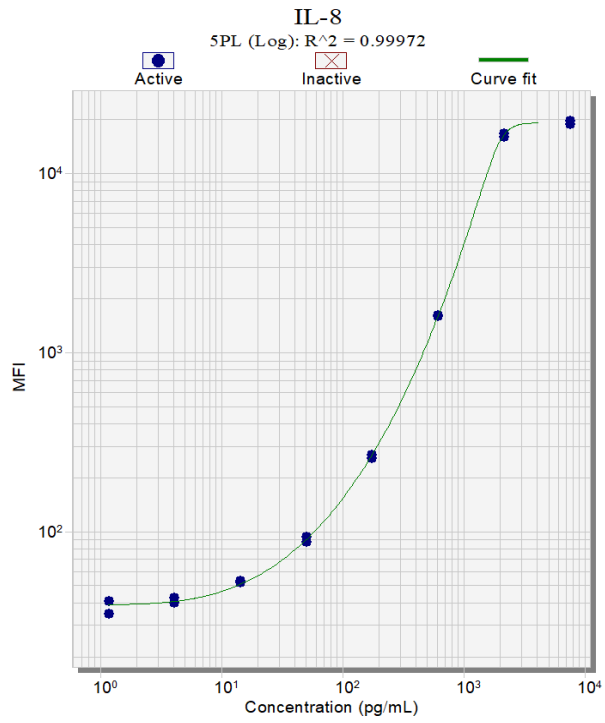
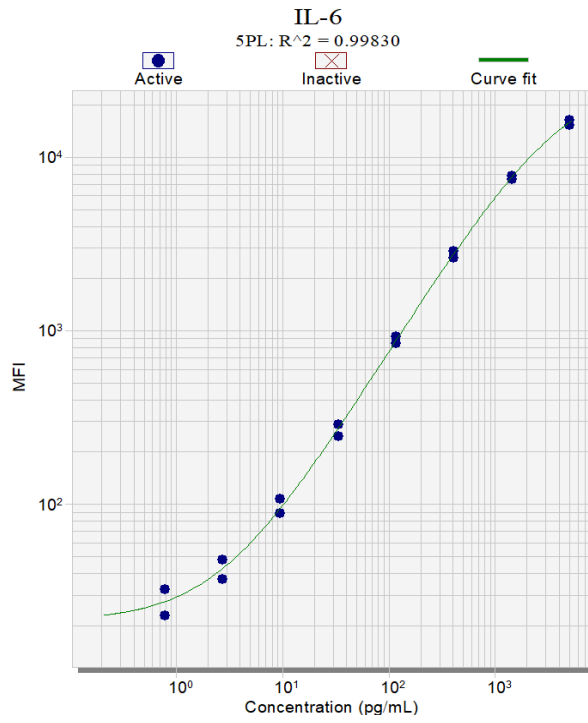
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in duplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

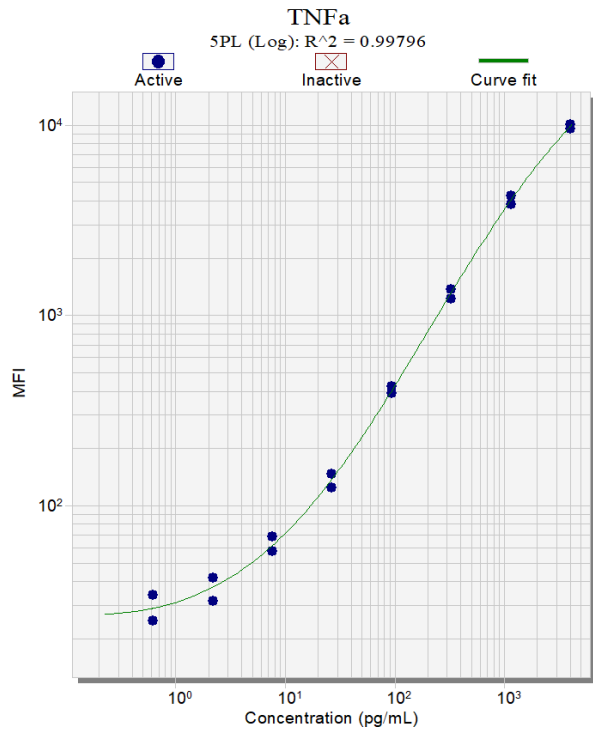
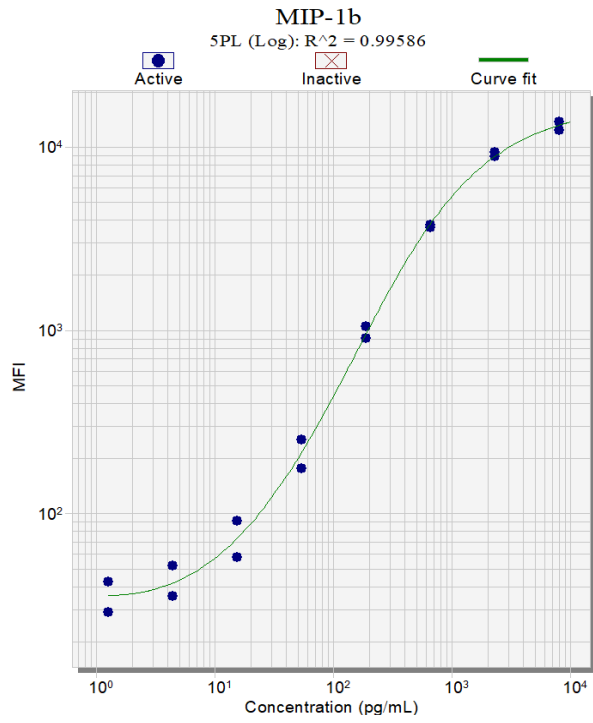
		S1	S8	LDD	LLOQ
IL-1 β	pg/ml	0.78	5000	3.3	3.3
IL-6	pg/ml	0.78	5000	0.78	2.2
IL-8	pg/ml	1.2	7500	5.5	5.8
MIP-1 β	pg/ml	1.2	8000	3.6	5.1
TNF α	pg/ml	0.62	4000	1.1	1.6

X Dilution Factor		S1	S8	LDD	LLOQ
IL-1 β	pg/ml	3.9	25000	16	16
IL-6	pg/ml	3.9	25000	3.9	11
IL-8	pg/ml	5.8	37500	27	29
MIP-1 β	pg/ml	6.2	40000	18	26
TNF α	pg/ml	3.1	20000	5.7	8.0

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.







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.

IL-1 β		1	2	3	Inter
Control 1	Mean	84	85	82	84
	% CV	2%	3%	2%	2%
Control 2	Mean	780	767	772	773
	% CV	1%	2%	2%	2%

IL-6		1	2	3	Inter
Control 1	Mean	86	86	84	86
	% CV	6%	2%	3%	4%
Control 2	Mean	941	942	885	923
	% CV	3%	8%	1%	5%

IL-8		1	2	3	Inter
Control 1	Mean	305	302	319	309
	% CV	1%	5%	2%	4%
Control 2	Mean	1573	1580	1667	1607
	% CV	1%	3%	1%	3%

MIP-1 β		1	2	3	Inter
Control 1	Mean	183	186	172	180
	% CV	1%	5%	4%	5%
Control 2	Mean	2173	2057	1893	2041
	% CV	3%	2%	2%	6%

TNF α		1	2	3	Inter
Control 1	Mean	58	59	54	57
	% CV	1%	1%	3%	4%
Control 2	Mean	430	409	396	412
	% CV	2%	8%	1%	6%

5. Linearity:

Linearity was assessed using 2 serum, 2 plasma 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.

		IL-1 β				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	Neat		216	124	200	138
	1:2		120	69	122	72
	1:4		65	26	65	37
	1:8		33	12	34	22
% Linearity	2		94%	86%	111%	102%
	4		90%	76%	119%	100%
	8		90%	73%	119%	114%

		IL-6				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	Neat		208	131	358	127
	1:2		106	66	159	72
	1:4		54	32	68	30
	1:8		27	15	32	15
% Linearity	2		102%	100%	89%	114%
	4		103%	99%	76%	94%
	8		103%	91%	72%	96%

		IL-8				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	Neat		329	882	312	771
	1:2		146	465	180	423
	1:4		79	231	78	242
	1:8		46	125	31	118
% Linearity	2		89%	105%	115%	110%
	4		96%	105%	99%	125%
	8		112%	113%	80%	123%

		MIP-1 β				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	Neat		664	188	371	28
	1:2		353	100	194	11
	1:4		173	50	99	LLOQ
	1:8		90	29	52	LLOQ
% Linearity	2		106%	107%	105%	79%
	4		104%	107%	107%	LLOQ
	8		109%	123%	113%	LLOQ

		TNF α				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	Neat		216	124	200	138
	1:2		120	69	122	72
	1:4		65	26	65	37
	1:8		33	12	34	22
% Linearity	2		113%	103%	115%	101%
	4		114%	90%	123%	115%
	8		122%	85%	120%	125%

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.

		IL-1 β				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		346	187	170	179
	FT-1X		353	197	164	184
	FT-2X		344	189	175	184
	FT-3X		348	197	168	186
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		102%	105%	96%	103%
	FT-2X		100%	101%	103%	103%
	FT-3X		101%	105%	99%	104%

		IL-6				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		328	197	355	217
	FT-1X		332	199	356	207
	FT-2X		324	192	359	224
	FT-3X		344	196	360	216
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		101%	101%	100%	96%
	FT-2X		99%	97%	101%	103%
	FT-3X		105%	99%	101%	100%

		IL-8				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		1260	1125	1430	1003
	FT-1X		1295	1120	1360	975
	FT-2X		1255	1029	1375	992
	FT-3X		1255	1095	1340	1010
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		103%	100%	95%	97%
	FT-2X		100%	91%	96%	99%
	FT-3X		100%	97%	94%	101%

		MIP-1 β				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		692	410	662	168
	FT-1X		706	419	650	171
	FT-2X		673	400	660	168
	FT-3X		690	416	673	168
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		102%	102%	98%	102%
	FT-2X		97%	98%	100%	100%
	FT-3X		100%	101%	102%	100%

		TNFα				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X		210	117	192	127
	FT-1X		210	114	188	130
	FT-2X		200	114	201	131
	FT-3X		205	120	198	138
% Control	FT-0X		100%	100%	100%	100%
	FT-1X		100%	97%	98%	103%
	FT-2X		95%	97%	104%	104%
	FT-3X		98%	102%	103%	109%

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.

8.

		IL-1 β				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR		312	183	178	160
	2hr RT		322	171	175	161
	2hr 4C		326	175	174	163
	4hr 4C		322	182	179	170
% Control	0 HR		100%	100%	100%	100%
	2hr RT		103%	94%	98%	101%
	2hr 4C		104%	96%	98%	102%
	4hr 4C		103%	100%	101%	106%

		IL-6				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR		315	187	364	196
	2hr RT		323	186	336	194
	2hr 4C		332	191	334	196
	4hr 4C		320	189	336	197
% Control	0 HR		100%	100%	100%	100%
	2hr RT		103%	99%	92%	99%
	2hr 4C		106%	102%	92%	100%
	4hr 4C		102%	101%	92%	101%

		IL-8				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR		921	856	1012	651
	2hr RT		966	804	1006	659
	2hr 4C		971	779	989	671
	4hr 4C		971	814	990	692
% Control	0 HR		100%	100%	100%	100%
	2hr RT		105%	94%	99%	101%
	2hr 4C		105%	91%	98%	103%
	4hr 4C		105%	95%	98%	106%

		MIP-1 β				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR		640	383	607	128
	2hr RT		649	374	603	126
	2hr 4C		667	370	579	123
	4hr 4C		671	383	593	131
% Control	0 HR		100%	100%	100%	100%
	2hr RT		101%	98%	99%	99%
	2hr 4C		104%	96%	95%	96%
	4hr 4C		105%	100%	98%	102%

		TNFα				
		pg/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR		198	120	187	121
	2hr RT		186	107	167	110
	2hr 4C		192	110	174	122
	4hr 4C		198	111	177	114
% Control	0 HR		100%	100%	100%	100%
	2hr RT		94%	89%	89%	90%
	2hr 4C		97%	92%	93%	100%
	4hr 4C		100%	92%	94%	94%