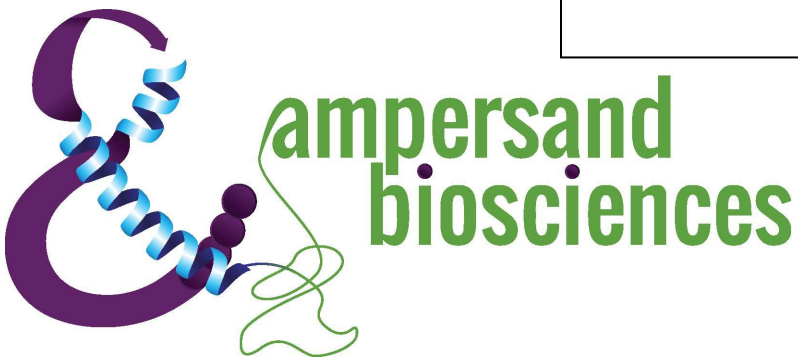
Human Cytokine Panel 4

Kit # HU121-K

Validation Report Version 1.0



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Reviewed by: Laurie Stephen Date 25-Jun-2025

1. Assay Description

A multiplex assay was developed and validated for CD155, MICA, MICB, ULBP1, ULBP2 and ULBP3 using the samples described in Section 2, which were prepared, aliquoted and stored at -80°C. Samples were run 1:5.

The kits are 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 serum is added to the appropriate wells. 10 µL of blocking buffer, followed by 10 µL multiplexed capture-antibody microspheres are then added to each well. The plate is sealed and incubated 1 hr at RT on a plate shaker. After washing, 40µL of biotinylated detection antibody is added to each well, thoroughly-mixed, and incubated for 1 hour on a plate shaker at RT. Without washing, 20 µL diluted streptavidin-phycoerythrin is added to each well, thoroughly-mixed, and incubated for 30 minutes. The plate is then washed 3 times and the beads are resuspended in 100 µL of sheath fluid. After shaking on a plate shaker for 5 min., the plate is then analyzed on the Luminex 100 Analyzer.

2. Control and Sample Description:

Control	Description
Control 1	Normal Human Plasma (10%) spiked with recombinant MICA, MICB, ULBP1, ULBP2, and ULBP3.
Control 2	Normal Human Plasma (20%) spiked with recombinant CD155, MICA, MICB, ULBP1, ULBP2, and ULBP3.

Sample	Description
Serum 1	SLE Human Serum spiked with recombinant MICA, MICB, ULBP1, and ULBP2.
Serum 2	Normal Human Serum spiked with recombinant MICA, ULBP1, ULBP2, and ULBP3.
Plasma 1	Normal Human Plasma spiked with recombinant CD155, MICA, MICB, ULBP1, ULBP2, Uland ULBP3.
Plasma 2	Normal Human Plasma spiked with recombinant MICA, MICB, ULBP1, ULBP2, and ULBP3.

3. LLOQ, LDD and Curves:

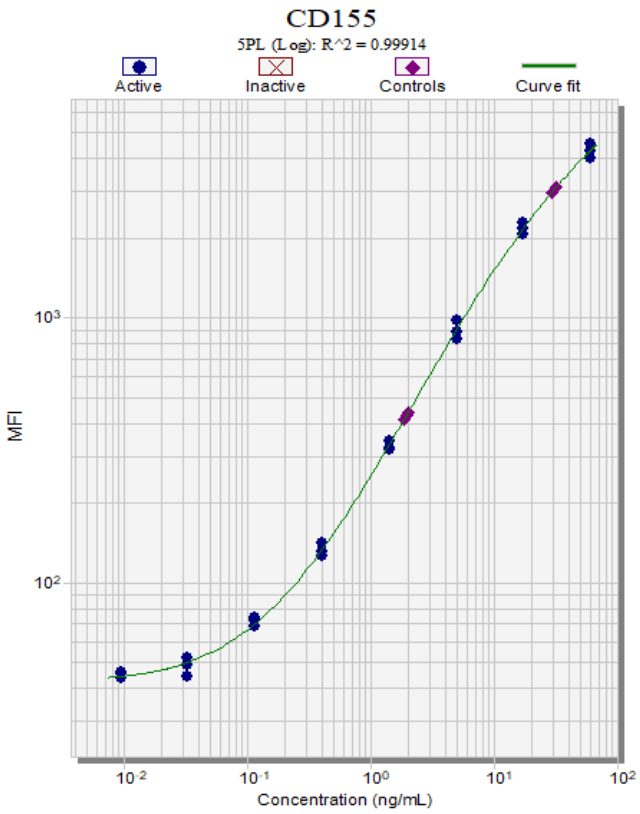
LDD: MFI (Median Fluorescent Intensity) for 8 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

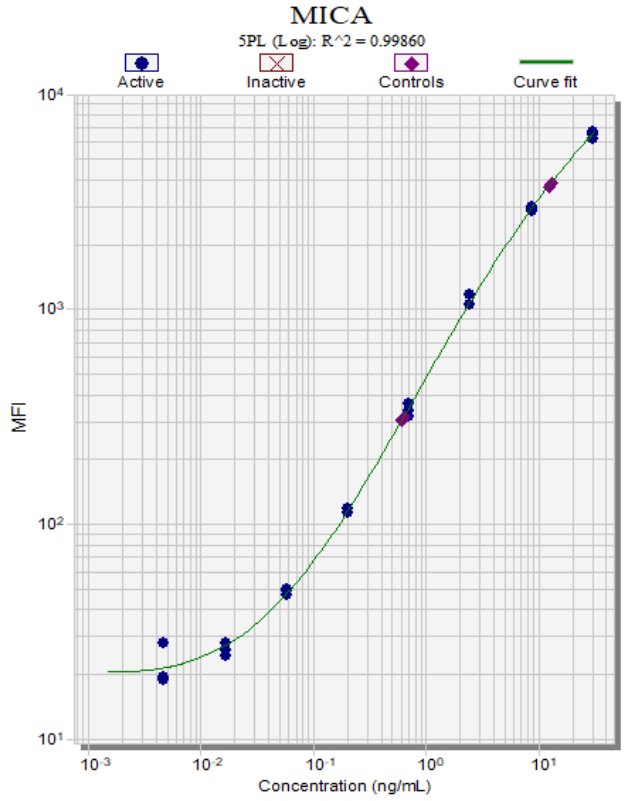
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in duplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

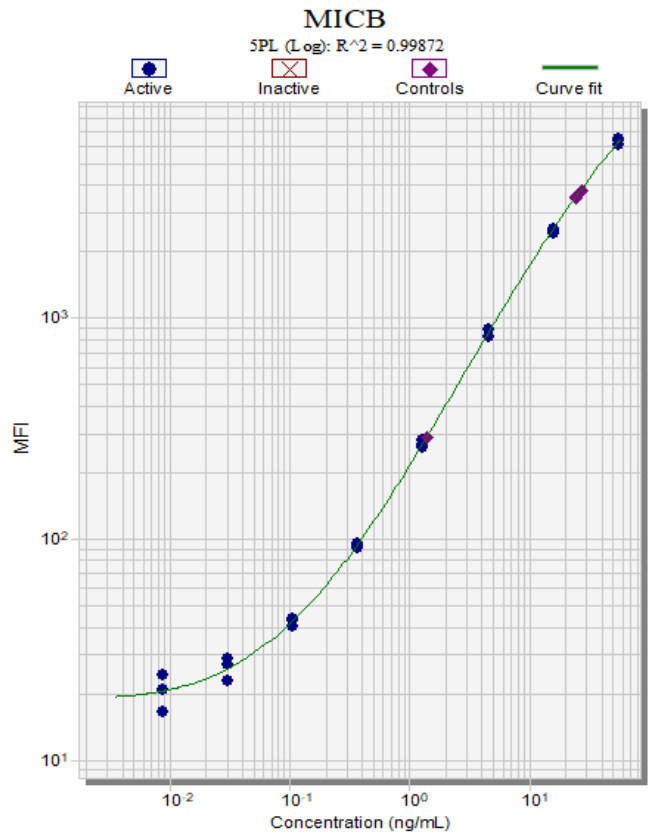
		S1	S8	LDD	LLOQ
CD155	**ng/mL**	0.0093	60	0.033	0.052
MICA	**ng/mL**	0.0047	30	0.0047	0.017
MICB	**ng/mL**	0.0086	55	0.012	0.032
ULBP1	**ng/mL**	0.0011	7.0	0.0030	0.0060
ULBP2	**ng/mL**	0.0070	45	0.021	0.028
ULBP3	**ng/mL**	0.00078	5.0	0.0020	0.0098

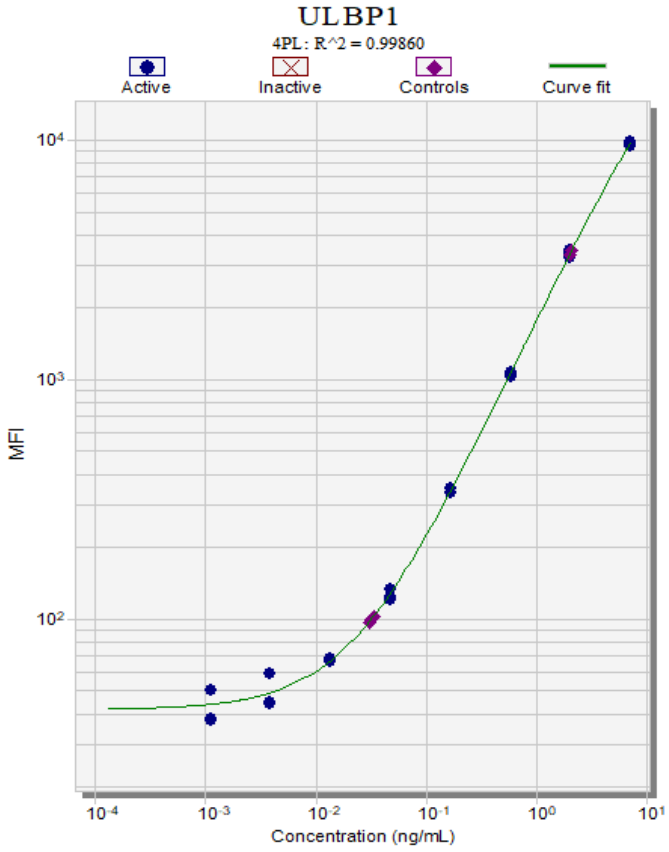
X Dilution Factor		S1	S8	LDD	LLOQ
CD155	**ng/mL**	0.047	300	0.16	0.26
MICA	**ng/mL**	0.023	150	0.023	0.085
MICB	**ng/mL**	0.043	275	0.059	0.16
ULBP1	**ng/mL**	0.0055	35	0.015	0.030
ULBP2	**ng/mL**	0.035	225	0.10	0.14
ULBP3	**ng/mL**	0.0039	25	0.010	0.049

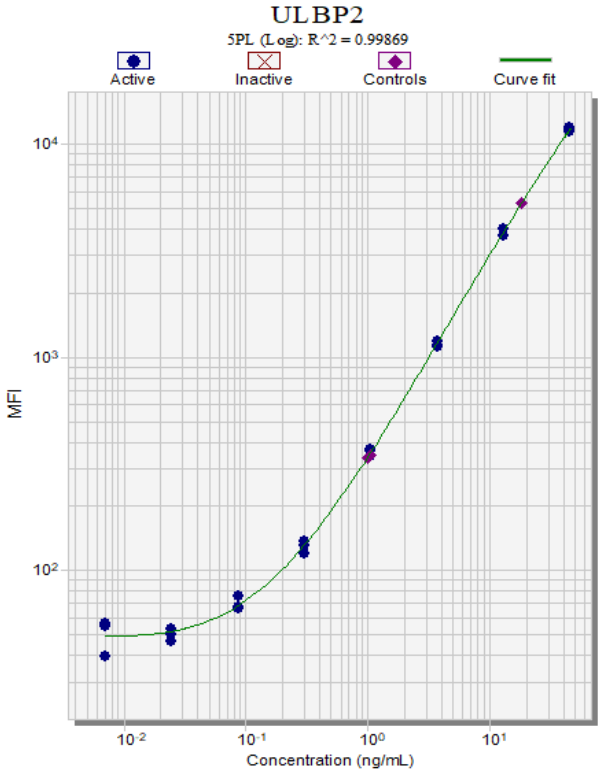
Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.

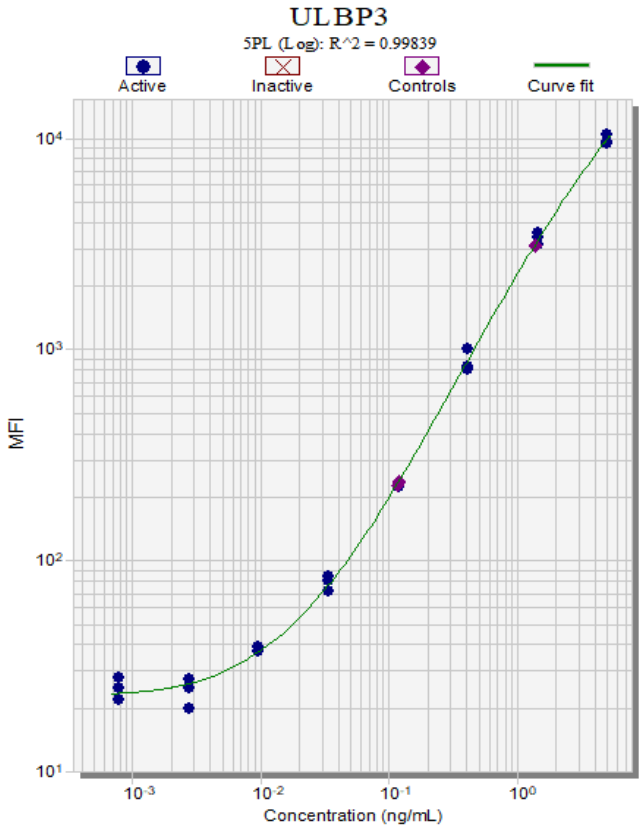

CD155
5PL (Log): R^2 = 0.99914
Active
Inactive
Controls
Curve fit
MFI
10³
10²
10⁻²
10⁻¹
10⁰
10¹
10²
Concentration (ng/mL)


MICA
5PL (Log): R^2 = 0.99860
Active
Inactive
Controls
Curve fit
MFI
10⁴
10³
10²
10¹
10⁻³
10⁻²
10⁻¹
10⁰
10¹
Concentration (ng/mL)


MICB
5PL (Log): R^2 = 0.99872
Active
Inactive
Controls
Curve fit
MFI
Concentration (ng/mL)


ULBP1
4PL: R^2 = 0.99860
Active
Inactive
Controls
Curve fit
MFI
Concentration (ng/mL)


ULBP2
5PL (Log): R^2 = 0.99869
Active
Inactive
Controls
Curve fit
MFI
Concentration (ng/mL)


ULBP3
5PL (Log): R^2 = 0.99839
Active
Inactive
Controls
Curve fit
MFI
Concentration (ng/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.

CD155		1	2	3	Inter
Control 1	**Mean**	1.9	2.0	1.9	**1.9**
	% CV	11%	18%	4%	**11%**
Control 2	**Mean**	30	34	30	**31**
	% CV	12%	1%	11%	**9%**

MICA		1	2	3	Inter
Control 1	**Mean**	0.53	0.47	0.61	**0.54**
	% CV	7%	5%	2%	**13%**
Control 2	**Mean**	12	11	13	**12**
	% CV	9%	10%	3%	**10%**

MICB		1	2	3	Inter
Control 1	**Mean**	1.2	1.0	1.4	**1.2**
	% CV	6%	8%	0%	**16%**
Control 2	**Mean**	22	22	25	**23**
	% CV	9%	4%	6%	**8%**

ULBP1		1	2	3	Inter
Control 1	**Mean**	0.025	0.026	0.032	**0.027**
	% CV	6%	10%	5%	**13%**
Control 2	**Mean**	2.1	2.0	2.0	**2.0**
	% CV	5%	1%	2%	**3%**

ULBP2		1	2	3	Inter
Control 1	**Mean**	1.0	0.90	1.0	**1.0**
	% CV	7%	0%	2%	**6%**
Control 2	**Mean**	18	15	18	**17**
	% CV	5%	3%	0%	**9%**

ULBP3		1	2	3	Inter
Control 1	**Mean**	0.11	0.11	0.12	**0.11**
	% CV	4%	6%	2%	**5%**
Control 2	**Mean**	1.3	1.3	1.4	**1.3**
	% CV	4%	1%	0%	**4%**

5. Linearity: Linearity was assessed by serially diluting samples 1:5 in sample diluent. Two fold dilutions were done from kit dilution on down to the LLOQ, testing % recovery from kit dilution vs. observed concentration. Average linearity was within the 70-130% acceptance for > 80 % of the samples for each given analyte.

	CD155				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	1:5	10	30	34	4.3
	1:10	6.4	14	16	2.6
	1:20	3.3	7.6	8.9	1.2
	1:40	1.6	3.2	4.4	0.51
% Linearity	10	122%	93%	95%	118%
	20	127%	99%	104%	107%
	40	121%	85%	103%	94%

	MICA				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	1:5	5.3	67	62	4.0
	1:10	2.3	35	28	2.0
	1:20	1.2	20	17	0.94
	1:40	0.59	8.2	7.5	0.51
% Linearity	10	88%	104%	88%	97%
	20	89%	117%	107%	93%
	40	88%	98%	96%	103%

	MICB				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	1:5	159	2.1	145	17
	1:10	84	1.2	67	9.1
	1:20	47	0.58	32	4.3
	1:40	23	0.22	18	1.9
% Linearity	10	107%	115%	92%	110%
	20	119%	110%	88%	103%
	40	117%	82%	97%	94%

	ULBP1				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	1:5	0.19	12	21	4.2
	1:10	0.10	6.6	10	1.8
	1:20	0.051	3.6	5.2	0.84
	1:40	0.020	1.7	2.7	0.49
% Linearity	10	103%	114%	92%	83%
	20	108%	122%	100%	80%
	40	86%	116%	105%	93%

	ULBP2				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	1:5	8.4	157	56	11
	1:10	4.5	79	23	5.3
	1:20	2.3	44	12	2.6
	1:40	1.1	19	6.1	1.3
% Linearity	10	107%	100%	84%	99%
	20	108%	113%	88%	97%
	40	108%	94%	87%	97%

	ULBP3				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	1:5	0.82	2.5	10	1.0
	1:10	0.39	1.5	4.3	0.50
	1:20	0.23	0.80	1.9	0.22
	1:40	0.12	0.39	0.96	0.14
% Linearity	10	96%	122%	88%	98%
	20	111%	128%	75%	89%
	40	120%	124%	78%	112%

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.

	CD155				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	10	35	24	9.1
	FT-1X	11	33	24	9.4
	FT-2X	11	35	22	10
	FT-3X	10	33	23	9.5
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	110%	93%	99%	104%
	FT-2X	115%	99%	92%	112%
	FT-3X	105%	93%	93%	104%

	MICA				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	1.5	74	49	5.8
	FT-1X	1.7	65	48	6.1
	FT-2X	1.7	75	47	6.2
	FT-3X	1.6	72	48	5.9
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	109%	88%	98%	106%
	FT-2X	112%	102%	96%	107%
	FT-3X	105%	97%	99%	101%

	MICB				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	68	3.2	156	16
	FT-1X	74	3.0	156	16
	FT-2X	83	3.1	148	16
	FT-3X	74	3.0	152	16
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	108%	95%	100%	102%
	FT-2X	122%	98%	95%	105%
	FT-3X	109%	95%	97%	105%

	ULBP1				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	0.15	12	21	6.1
	FT-1X	0.17	12	21	6.2
	FT-2X	0.17	12	20	6.4
	FT-3X	0.15	11	20	6.4
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	117%	96%	104%	103%
	FT-2X	120%	101%	97%	105%
	FT-3X	102%	92%	100%	106%

	ULBP2				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	2.8	157	39	11
	FT-1X	2.8	133	38	12
	FT-2X	3.0	158	37	12
	FT-3X	3.2	149	38	12
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	99%	85%	99%	104%
	FT-2X	107%	101%	96%	104%
	FT-3X	111%	95%	99%	101%

	ULBP3				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	FT-0X	0.17	3.7	14	1.5
	FT-1X	0.20	3.3	14	1.5
	FT-2X	0.20	3.7	13	1.5
	FT-3X	0.18	3.4	14	1.5
% Control	FT-0X	100%	100%	100%	100%
	FT-1X	117%	91%	102%	103%
	FT-2X	118%	101%	92%	103%
	FT-3X	106%	92%	101%	107%

7. Bench-top stability: Samples were assessed for bench top stability at 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.

	CD155				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	12	33	30	11
	2hr RT	15	33	26	10
	2hr 4C	12	32	31	10
	4hr 4C	12	36	26	11
% Control	0 HR	100%	100%	100%	100%
	2hr RT	123%	100%	85%	95%
	2hr 4C	100%	96%	101%	96%
	4hr 4C	98%	110%	86%	100%

	MICA				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	1.6	68	61	6.9
	2hr RT	1.8	57	60	6.6
	2hr 4C	1.7	65	63	6.9
	4hr 4C	1.8	73	57	7.2
% Control	0 HR	100%	100%	100%	100%
	2hr RT	111%	84%	98%	95%
	2hr 4C	103%	96%	102%	99%
	4hr 4C	112%	107%	93%	103%

	MICB				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	79	3.2	199	18
	2hr RT	85	2.8	200	18
	2hr 4C	76	3.6	202	19
	4hr 4C	77	3.6	183	18
% Control	0 HR	100%	100%	100%	100%
	2hr RT	108%	87%	101%	98%
	2hr 4C	97%	111%	102%	105%
	4hr 4C	98%	112%	92%	100%

	ULBP1				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	0.28	12	23	7.1
	2hr RT	0.29	12	23	6.8
	2hr 4C	0.28	11	24	7.2
	4hr 4C	0.30	12	22	7.5
% Control	0 HR	100%	100%	100%	100%
	2hr RT	102%	101%	99%	96%
	2hr 4C	98%	95%	103%	102%
	4hr 4C	107%	98%	92%	105%

	ULBP2				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	3.1	141	49	14
	2hr RT	3.5	132	49	12
	2hr 4C	3.2	134	50	13
	4hr 4C	3.1	149	45	14
% Control	0 HR	100%	100%	100%	100%
	2hr RT	112%	94%	100%	85%
	2hr 4C	105%	95%	101%	94%
	4hr 4C	99%	106%	92%	97%

	ULBP3				
	ng/ml	Serum 1	Serum 2	Plasma 1	Plasma 2
Value	0 HR	1.1	3.3	16	1.7
	2hr RT	1.0	3.3	16	1.5
	2hr 4C	1.1	3.6	16	1.7
	4hr 4C	1.2	3.5	14	1.7
% Control	0 HR	100%	100%	100%	100%
	2hr RT	90%	99%	102%	90%
	2hr 4C	94%	108%	102%	96%
	4hr 4C	107%	106%	91%	100%