IL-6 ELISA

Cat. No. BI-IL6 12x8 Tests

IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN INTERLEUKIN-6 (IL-6) IN SERUM, PLASMA, CELL CULTURE SUPERNATANTS, AND URINE

For research use only. Not for use in diagnostic procedures.

CONTENTS

ASSAY CHARACTERISTICS OVERVIEW

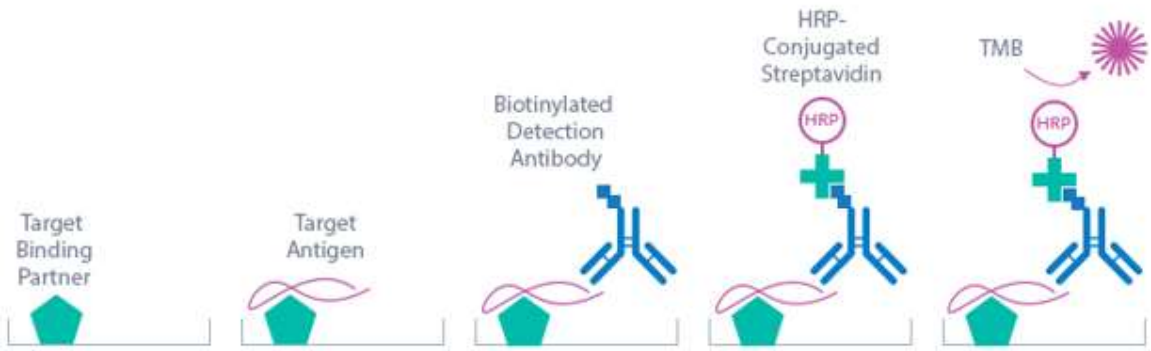
Method	Sandwich ELISA, HRPO/TMB, 12x8-well detachable strips				
Sample type(s)	Serum, plasma (EDTA, citrate, heparin), cell culture supernatants, urine				
Sample volume	100 µl sample / well				
Standard range	0 – 200 pg/ml (0 / 3.12 / 6.25 / 12.5 / 25 / 50 / 100 / 200)				
Sensitivity	LOD: 0.28 pg/ml; LLOQ: 0.78 pg/ml *(measurable concentrations in serum AND plasma samples!)*				
Assay time	2 h / 1 h / 1h / 30 min				
Conversion	1 pg/ml=0.048 pmol/l (MW=20.8 kD)				
Precision		**n**	**Average % CV**		
	Within-run	3	≤7		
	In-between-run		*In progress*		
Accuracy (Spike/Recovery of recombinant human IL-6)		**n**	**Average % recovery**		
			+100 pg/ml	**+50 pg/ml**	
	Serum	6	113	112	
	EDTA plasma	6	111	109	
	Citrate plasma	2	111	99	
	Heparin plasma	2	107	103	
	Cell culture	3	n.d.	97	
	Urine	5	n.d.	102	
Parallelism of endogenous human IL-6		**n**	**Average % of expected dilution**		
			1+1	**1+3**	**1+7**
	Serum	5	93	90	89
	EDTA plasma	5	99	96	92
	Citrate plasma	2	103	100	99
	Heparin plasma	2	104	90	89
	Cell culture	2	95	102	98
	Urine	2	96	108	108
Specificity	This assay recognizes recombinant and endogenous (natural) human IL-6.				
Use	Research use only.				
Values of apparently healthy donors		**n**	**Median / Range IL-6 pg/ml**		
	Serum	48	1.50 (0.30-4.36)		
	EDTA plasma	26	0.98 (0.01-2.69)		
	Citrate plasma	14	0.71 (0.01-2.10)		
	Heparin plasma	11	0.60 (0-2.41)		
	Urine	4	0.77 (0-1.5)		

Abbreviation: n.d.: not determined

ASSAY PRINCIPLE

The Biomedica human Interleukin-6 ELISA (IL-6) ELISA kit is a sandwich enzyme immunoassay that has been optimized and fully validated for the quantitative determination of human IL-6 in serum and plasma (EDTA, heparin, citrate). Validation experiments have been performed according to international quality guidelines (ICH/ FDA/ EMEA). Cell culture supernatant and urine samples are compatible with this ELISA (data download: www.bmgrp.com). The IL-6 ELISA assay recognizes both natural and recombinant human IL-6. The assay employs highly purified epitope mapped antibodies as well as human serum-based standards and controls.

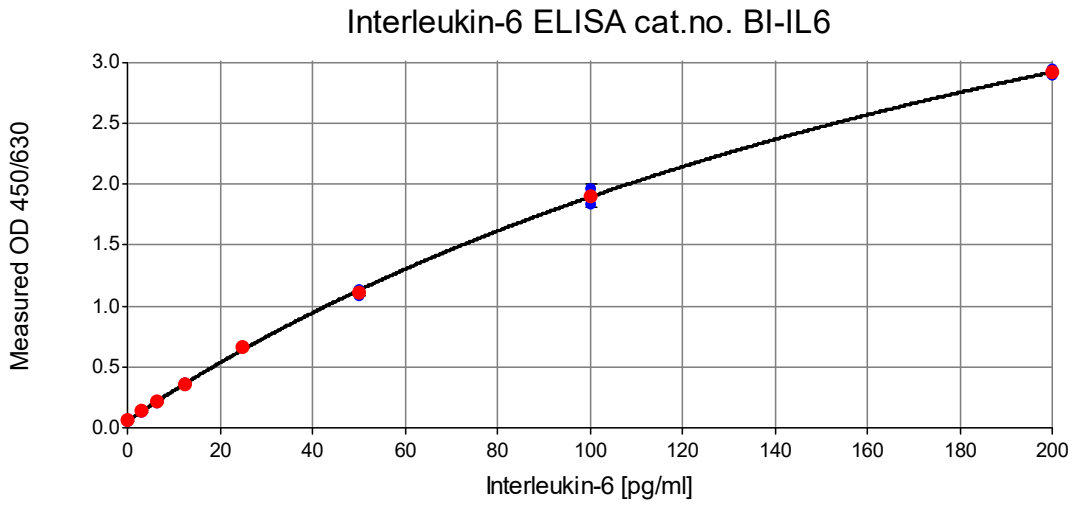
The figure below explains the principle of the human IL-6 sandwich ELISA:



In a first step, STD/sample/CTRL are pipetted into the wells, which are pre-coated with the recombinant anti-human IL-6 antibody. Any soluble IL-6 present in the STD/sample/CTRL binds to the pre-coated anti-IL-6 antibody in the well. After incubation, a washing step is applied where all non-specific unbound material is removed. In a next step, the biotinylated anti-IL-6 antibody (AB) is pipetted into the wells and reacts with the IL-6 present in the sample, forming a sandwich. Next, all unbound antibody is removed during another washing step. In the next step, the conjugate (streptavidin-HRPO) is added and reacts with the biotinylated anti-IL-6 antibody. After another washing step, the substrate (Tetramethylbenzidine; TMB) is pipetted into the wells. The enzyme catalysed color change of the substrate is directly proportional to the amount of IL-6 present in the sample. This color change is detectable with a standard microtiter plate ELISA reader. A dose response curve of the absorbance (optical density, OD at 450 nm) versus standard concentration is generated, using the values obtained from the standards. The concentration of soluble IL-6 in the sample is determined directly from the dose response curve.

TYPICAL DATA

This standard curve and the displayed OD values are for demonstration only. A standard curve should be generated for each assay run.



Standard	IL-6 [pg/ml]	OD			CV [%]
		#1	#2	Average	
STD1	0	0.056	0.059	**0.058**	4
STD2	3.125	0.140	0.129	**0.135**	6
STD3	6.25	0.216	0.207	**0.212**	3
STD4	12.5	0.354	0.366	**0.360**	2
STD5	25	0.661	0.660	**0.661**	0
STD6	50	1.090	1.138	**1.114**	3
STD7	100	1.970	1.835	**1.903**	5
STD8	200	2.951	2.894	**2.923**	1

The quality control protocol supplied with the kit shows the results of the final release QC for each kit at the production date. ODs obtained by customers may differ due to various influences including a normal decrease of signal intensity throughout shelf life. However, this does not affect the validity of the results, provided an OD of 1.50 or higher is obtained for the standard with the highest concentration, and the measured control values fall into their respective target range (see labels).

CALIBRATION

The Biomedica human Interleukin-6 (IL-6) ELISA kit is calibrated against a highly purified recombinant human IL-6 protein (expressed in human embryonic kidney cell, HEK-293).
The human serum based calibrator is provided in eight lyophilized glass vials in the following concentrations: 0 / 3.125 / 6.25 / 12.5 / 25 / 50 / 100 / 200 pg/ml.

CALIBRATION using WHO standard
The WHO reference reagent IL-6/NIBSC code 89/548 (recombinant DNA, human sequence) was analysed in this human IL-6 ELISA kit.

The equation below can be used to convert the sample values obtained with this kit to approximate WHO/IL-6 /NIBSC 89/548 units:

WHO/NIBSC (89/548) reference (IU/ml) = 0.08 BI-IL6 value (pg/ml).

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the IL-6 ELISA, experiments measuring the Lower Limit of Detection (LOD) and the Lower Limit of Quantification (LLOQ) were conducted.

The LOD, also called the detection limit, is the lowest point at which a signal can be distinguished from the background signal, *i.e.*, the signal that is measured in the absence of IL-6, with a confidence level of 99%. It is defined as the mean back-calculated concentration of standard 1 (0 pg/ml of IL-6, five independent measurements) plus three times the standard deviation of the measurements.

The LLOQ, or sensitivity of an assay, is the lowest concentration at which an analyte can be accurately quantified. The criteria for accurate quantification at the LLOQ are an analyte recovery between 75% and 125% and a coefficient of variation (CV) of less than 25%. To determine the LLOQ, standard 2, *i.e.*, the lowest standard containing IL-6, is diluted, measured five times and its concentration back calculated. The lowest dilution, which meets both criteria, is reported as the LLOQ.

The following values were determined for the human IL-6 ELISA:

LOD	0.28 pg/ml
LLOQ	0.78 pg/ml

PRECISION

The precision of an ELISA is defined as its ability to measure the same concentration consistently within the same experiments carried out by one operator (within-run precision or repeatability) and across several experiments using the same samples but conducted by several operators at different locations using different ELISA lots (in-between-run precision or reproducibility).

Within-Run Precision

Within-run precision was tested by measuring two samples of known concentrations three times within one IL-6 ELISA lot by one operator.

ID	n	Mean IL-6 [pg/ml]	SD IL-6 [pg/ml]	CV [%]
Sample 1	3	6.5	0.5	7
Sample 2	3	50.5	0.7	1

In-Between-Run Precision

In progress

ACCURACY

The accuracy of an ELISA is defined as the precision with which it can recover samples of known concentrations.

The recovery of the human Interleukin-6 (IL-6) ELISA was measured by adding recombinant human IL-6 to samples containing a known concentration of endogenous IL-6. The %recovery of the spiked concentration was calculated as the percentage of measured over the expected value.

This table shows the summary of the recovery experiments in the IL-6 ELISA in different sample matrices:

Sample Matrix	n	% Recovery [recombinant IL-6]]					
		+100 pg/ml		+50 pg/ml		+25 pg/ml	
		Mean	Range	Mean	Range	Mean	Range
Serum	6	113	103-122	112	103-120	96	88-103
EDTA plasma	6	111	107-116	109	103-113	99	93-104
Citrate plasma	2	111	106-115	99	97-102	102	99-105
Heparin plasma	2	107	105-109	103	103-104	95	89-100
Cell culture supernatant	2	n.d.	n.d.	97	90-103	-	-
Urine	5	n.d.	n.d.	102	95-110	-	-

n.d.: not determined

Experiments:
Recovery of spiked samples was tested by adding various concentrations of human recombinant IL-6 to different human sample matrices.

Data showing % recovery of recombinant IL-6 in human serum samples:

ID	IL-6				% Recovery		
	Reference	+100 pg/ml	+50 pg/ml	+25 pg/ml	+100 pg/ml	+50 pg/ml	+25 pg/ml
S1	2.7	104.4	52.8	26.6	103	103	97
S2	2.5	114.1	53.7	24.8	113	105	90
S3	1.4	119.7	57.6	26.1	119	114	99
S4	2.5	113.7	61.1	26.4	112	120	97
S5	0.5	122.3	56.9	26.2	122	113	103
S6	17.7	116.2	66.8	37.6	107	116	88
				Mean R[%]	**113**	**112**	**96**

Data showing % recovery of recombinant IL-6 in human EDTA plasma samples:

ID	IL-6				% Recovery		
	Reference	+100 pg/ml	+50 pg/ml	+25 pg/ml	+100 pg/ml	+50 pg/ml	+25 pg/ml
E1	2.1	110.7	57.4	26.1	110	113	97
E2	2.7	116.9	52.8	25.5	116	103	93
E3	0.7	107.6	55.5	25.3	107	110	99
E4	2.9	113.4	56.0	27.2	112	109	99
E5	0.6	111.1	55.2	26.4	111	110	104
E6	0.3	110.1	55.4	25.5	110	111	101
				Mean R[%]	**111**	**109**	**99**

Data showing % recovery of recombinant IL-6 in human citrate plasma samples:

ID	IL-6				% Recovery		
	Reference	+100 pg/ml	+50 pg/ml	+25 pg/ml	+100 pg/ml	+50 pg/ml	+25 pg/ml
C1	2.1	115.6	52.2	28.1	115	102	105
C2	1.7	107.3	49.1	26.3	106	97	99
				Mean R[%]	**111**	**99**	**102**

Data showing % recovery of recombinant IL-6 in human heparin plasma samples:

ID	IL-6				% Recovery		
	Reference	+100 pg/ml	+50 pg/ml	+25 pg/ml	+100 pg/ml	+50 pg/ml	+25 pg/ml
H1	0.9	105.8	52.2	25.8	105	104	100
H2	0.9	109.4	51.7	23.1	109	103	89
				Mean R[%]	**107**	**103**	**95**

DILUTION LINEARITY & PARALLELISM

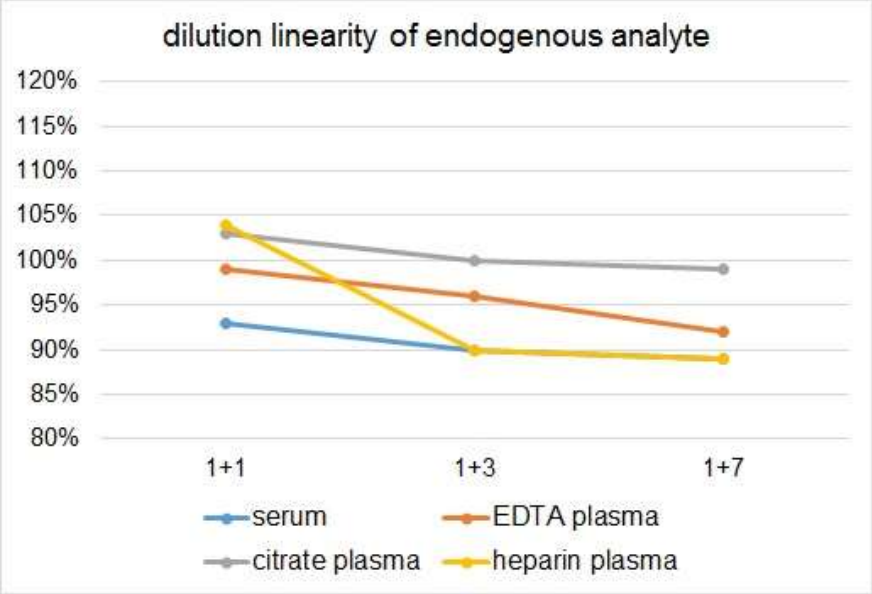
Tests of dilution linearity and parallelism ensure that both, endogenous and recombinant samples containing IL-6 behave in a dose-dependent manner and are not affected by matrix effects. Dilution linearity assesses the accuracy of measurements in diluted human samples spiked with known concentrations of recombinant analyte. By contrast, parallelism refers to dilution linearity in clinical samples and provides evidence that endogenous analyte behaves in the same way as the recombinant one /likewise to the recombinant analyte. Dilution linearity and parallelism are assessed for each sample type and should be within 20% of the expected concentration.

Parallelism

Experiment:
Parallelism was assessed by serially diluting human samples containing **endogenous** IL-6 with assay buffer.

Summary table below shows the mean recovery and range of serially diluted endogenous IL-6 in several sample matrices:

Sample Matrix	n	% Recovery of endogenous IL-6 in diluted samples					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Serum	5	93	82-100	90	86-94	89	84-93
EDTA plasma	5	99	92-104	96	76-114	92	86-98
Citrate plasma	2	103	101-106	100	92-109	99	92-106
Heparin plasma	2	104	104-104	90	84-95	89	88-90
Cell culture supernatant	2	95	89-101	102	88-117	98	88-108
Urine	2	96	95-98	108	97-120	108	93-124



Data showing dilution linearity of endogenous IL-6 in human serum samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
S1	40.3	19.4	9.1	4.7	96	90	93
S2	44.4	18.1	9.9	4.9	82	89	88
S3	91.9	41.6	21.5	10.0	91	94	87
S4	39.9	20.0	9.1	4.2	100	91	84
S5	45.8	22.3	9.6	5.2	97	86	91
				Mean R[%]	**93**	**90**	**89**

Data showing dilution linearity of endogenous IL-6 in human EDTA plasma samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
E1	74.2	34.1	14.1	8.2	92	76	88
E2	22.0	11.0	5.2	2.7	100	95	98
E3	38.1	18.1	8.8	4.1	95	92	86
E4	9.8	5.0	2.8	1.1	102	114	90
E5	31.4	16.3	8.0	3.8	104	102	97
				Mean R[%]	**99**	**96**	**92**

Data showing recovery of endogenous IL-6 in human citrate plasma samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
C1	59.3	29.9	13.7	6.8	101	92	92
C2	147.8	78.2	40.1	19.5	106	109	106
				Mean R[%]	**103**	**100**	**99**

Data showing recovery of endogenous IL-6 in human heparin plasma samples:

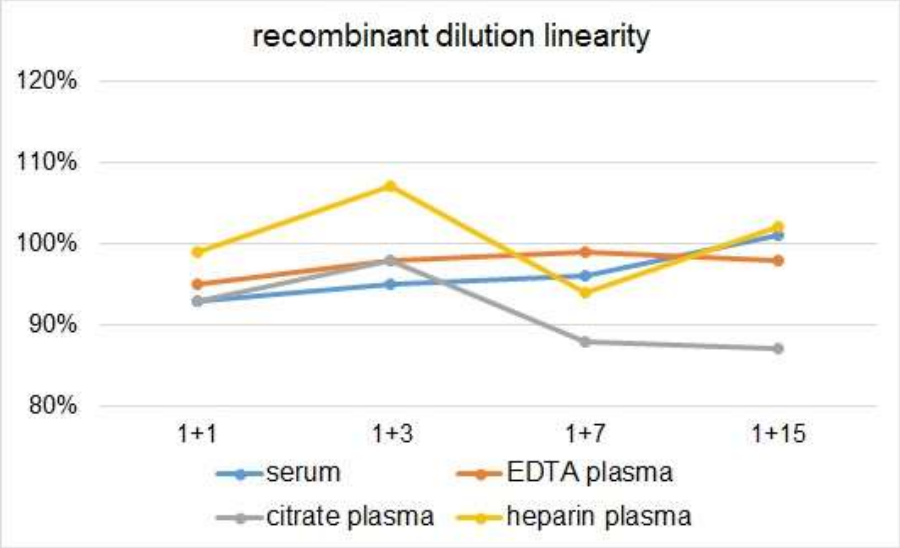
ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
H1	144.7	75.1	34.5	16.0	104	95	88
H2	92.1	48.0	19.4	10.4	104	84	90
				Mean R[%]	**104**	**90**	**89**

Dilution Linearity

Experiment:
Dilution linearity was assessed by serially diluting samples containing **recombinant** human IL-6 with assay buffer.

The figure and table below show the mean recovery and range of serially diluted recombinant IL-6 in several sample matrices:

Sample Matrix	n	% Recovery of recombinant IL-6 in diluted samples					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Serum	5	93	83-100	95	85-104	96	91-104
EDTA plasma	5	95	85-104	98	93-104	99	94-106
Citrate plasma	2	93	93-94	98	94-102	88	86-89
Heparin plasma	2	99	94-104	107	103-111	94	94-94
Cell culture supern	2	93	90-97	95	92-97	96	94-98
Urine	1	89	-	99	-	89	-



Data showing dilution linearity of recombinant IL-6 spiked into human serum and plasma samples (reference) containing endogenous IL-6.

Calculation of dilution linearity of spiked serum samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
S1	126.8	63.1	30.9	14.7	100	97	93
S2	114.1	50.6	26.3	14.8	89	92	104
S3	119.7	57.7	29.0	14.3	96	97	96
S4	113.7	54.7	29.7	14.3	96	104	96
S5	124.7	51.6	26.4	14.2	83	85	91
				Mean R[%]	**93**	**95**	**96**

Calculation of dilution linearity of spiked EDTA plasma samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
E1	110.7	57.8	28.9	14.7	104	104	106
E2	116.9	49.5	28.3	14.2	85	97	97
E3	111.1	50.4	25.8	13.4	91	93	96
E4	111.4	52.7	26.0	14.1	95	93	101
E5	119.3	60.7	30.7	14.0	102	103	94
				Mean R[%]	**95**	**98**	**99**

Calculation of dilution linearity of spiked citrate plasma samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
C1	115.6	54.2	29.4	12.9	94	102	89
C2	107.3	49.9	25.2	11.5	93	94	86
				Mean R[%]	**93**	**98**	**88**

Calculation of dilution linearity of spiked heparin plasma samples:

ID	IL-6 [pg/ml]				% Recovery		
	Reference	1+1	1+3	1+7	1+1	1+3	1+7
H1	105.8	49.6	27.2	12.4	94	103	94
H2	109.4	56.8	30.3	12.9	104	111	94
				Mean R[%]	**99**	**107**	**94**

SPECIFICITY

The specificity of an ELISA is defined as its ability to exclusively recognize the analyte of interest.

The specificity of the IL-6 ELISA was shown by characterizing both the capture and the detection antibodies through epitope mapping. In addition, the specificity of the ELISA was established through competition experiments, which measure the ability of the antibodies to exclusively bind IL-6.

> *This assay recognizes recombinant and endogenous (natural) human IL-6 and detects free circulating IL-6 as well as receptor-bound IL-6.*

The recombinant anti-human IL-6 capture antibody detects a structural epitope near the receptor binding site of the IL-6 molecule. Four linear epitopes of the polyclonal anti-human IL-6 detection antibody are spread throughout the IL-6 protein.

Competition of Signal

Competition experiments were carried out by pre-incubating human samples containing endogenous IL-6 with an excess of capture antibody (AB). The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample without the pre-incubation step. Mean competition in serum and plasma samples is 99%.

Sample Matrix	ID	IL-6 [pg/ml]		% Competition
		Reference	+ capture AB	
Serum	S1	1.4	0.0	95
Serum	S2	61.4	0.3	99
Serum	S3	41.5	0.3	99
Serum	S4	33.2	0.6	98
EDTA plasma	E1	18.9	0.2	99
EDTA plasma	E2	27.6	0.1	99
Citrate plasma	C1	45.6	0.0	100
Heparin plasma	H1	81.0	0.0	100
			Mean R[%]	**99**

CROSS REACTIVITY with non-human samples

This human IL-6 ELISA kit cannot be used for the detection of IL-6 in rat, mouse, and porcine samples.

SAMPLE STABILITY

Serum, EDTA plasma, heparin plasma, citrate plasma, cell culture supernatants, and urine samples are suitable for use in this assay. Do not change sample type during studies. We recommend duplicate measurements for all samples, standards and controls. The sample collection and storage conditions listed are intended as general guidelines.

Freeze-thaw Stability

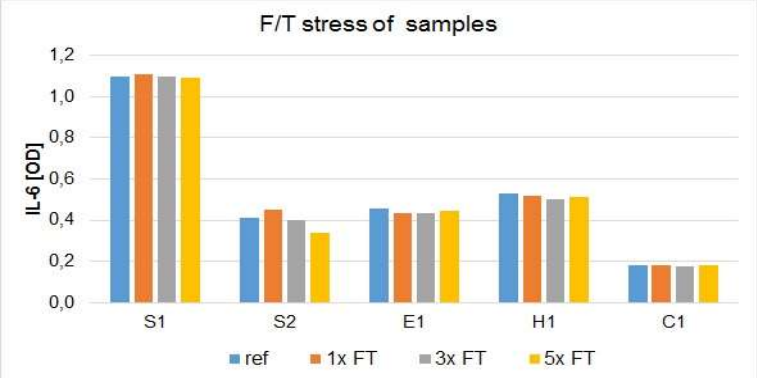
The stability of endogenous Interleukin-6 (IL-6) was tested by comparing samples that had undergone five freeze-thaw cycles (F/T).

For freeze-thaw experiments, samples were collected according to the supplier's instruction using blood collection devices and stored at -80°C. Reference samples were freeze-thawed once. The mean recovery of sample concentration after five freeze-thaw cycles is 95%.

Sample OD values after freeze-thaw (F/T) cycles:

Sample Matrix	ID	IL-6 [OD]				% Recovery		
		Reference	1x F/T	3x F/T	5x F/T	1x F/T	3x F/T	5x F/T
Serum	S1	1.09	1.10	1.09	1.09	101	100	100
Serum	S2	0.41	0.45	0.43	0.33	110	98	82
EDTA plasma	E1	0.45	0.43	0.43	0.44	95	95	98
Citrate plasma	C1	0.18	0.18	0.17	0.18	98	95	99
Heparin plasma	H1	0.53	0.52	0.50	0.51	98	94	97
					Mean R[%]	**101**	**97**	**95**

All samples should undergo a maximum of five freeze-thaw cycles.



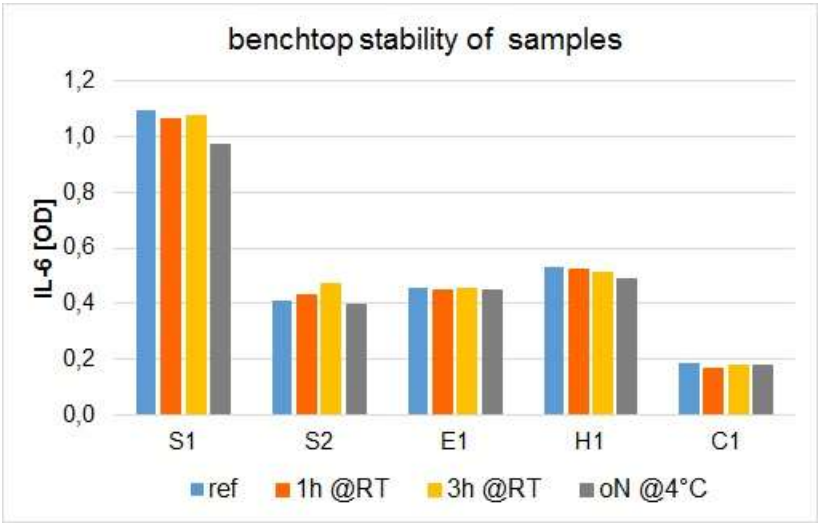
Benchtop Stability

The benchtop stability of endogenous Interleukin-6 (IL-6) was tested by comparing IL-6 measurements in human samples that had been stored at different temperatures.

For the assessment of the benchtop stability, a set of human samples was aliquoted and stored at room temperature or at 4°C. Samples can be stored for at least three hours at room temperature as well as overnight at 4°C. The mean recovery of sample concentrations after overnight storage at 4°C is 95%.

Sample OD values of samples stored at -25°C (reference), at room temperature (RT) or overnight (o.n.) at 4°C:

		IL-6 (OD)			% Recovery	
Sample Matrix	**ID**	**Reference**	**3h @RT**	**o.n. @4°C**	**3h @RT**	**o.n. @4°C**
Serum	S1	1.09	1.07	0.97	98	89
Serum	S2	0.41	0.46	0.39	114	97
EDTA plasma	E1	0.45	0.45	0.44	99	98
Citrate plasma	C1	0.18	0.17	0.17	96	97
Heparin plasma	H1	0.53	0.51	0.49	96	92
				Mean R [%]	**101**	**95**



SAMPLE VALUES

IL-6 Values in Apparently Healthy Individuals

To provide values for circulating Interleukin-6 (IL-6), a panel of samples from apparently healthy donors was tested (no medical histories were available). Each individual donated blood for all tested sample matrices.

		IL-6 [pg/ml]			% Detectable
Sample Matrix	**n**	**Mean**	**Range**	**Median**	
Serum	48	1.73	0.30 – 4.36	1.50	100
EDTA plasma	26	1.01	0.01 – 2.69	0.98	100
Citrate plasma	14	1.86	0.01 – 2.10	0.71	100
Heparin plasma	11	1.52	0.00 – 2.41	0.60	91

It is recommended to establish the normal range for each laboratory.

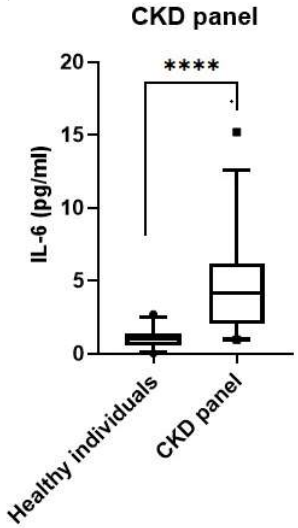
IL-6 values measured in serum and plasma (EDTA, citrate, heparin) samples of ten apparently healthy donors (matched-paired samples):

		IL-6 [pg/ml]			% Detectable
Sample Matrix	**n**	**Mean**	**Range**	**Median**	
Serum	10	1.12	0.58-1.56	1.19	100
EDTA plasma	10	0.64	0.01-1.45	0.53	100
Citrate plasma	10	0.71	0.01-1.32	0.68	100
Heparin plasma	9	0.62	0.00-2.41	0.48	90

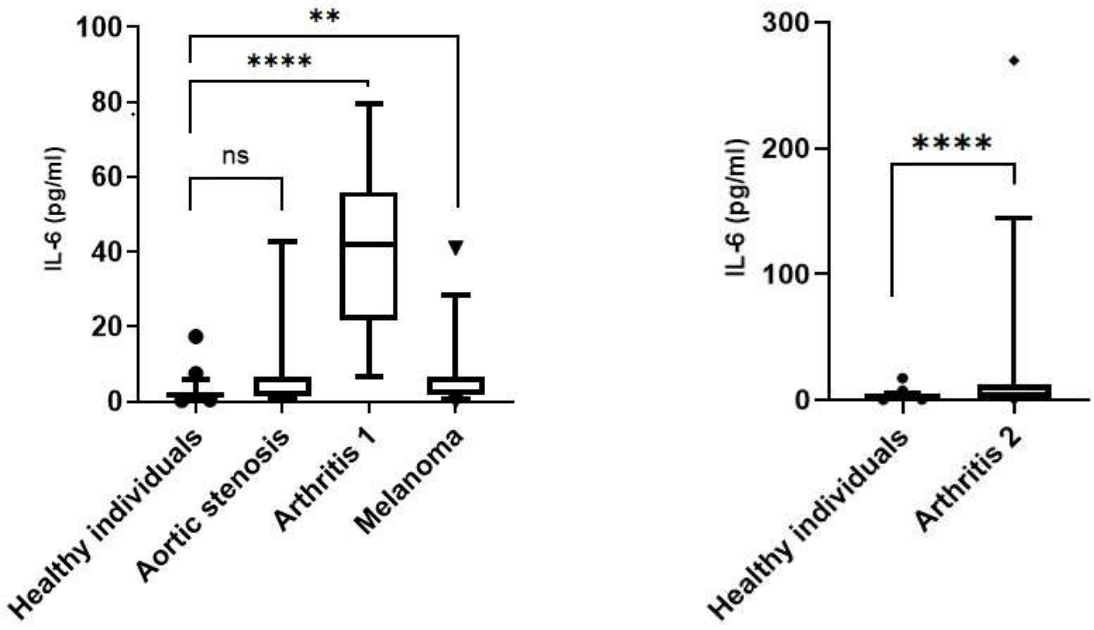
IL-6 Values in Disease Panels

In addition to samples of apparently healthy donors, panels of samples from patients with heart disease (aortic stenosis), kidney diseases, as well as a panel of unselected hospital patients were tested.

IL-6 values measured in apparently healthy individuals and in subjects with chronic kidney disease (CKD).



IL-6 values measured in apparently healthy individuals and in subjects with aortic stenosis, arthritis, and melanoma.



Summary of the results:

		IL-6 [pg/ml]		
Samples / Matrix	**n**	**Mean**	**Range**	**Median**
Apparently Healthy Panel / Serum	48	1.7	0.3-4.3	1.5
Aortic Stenosis Panel / Serum	18	6.5	0.8-42.6	2.0
Arthritis Panel I / Serum	13	40.5	6.5-79.4	41.9
Arthritis Panel II / Serum	32	18.5	0.4-270	3.4
Melanoma Panel / Serum	32	6.0	0-40.9	2.5
Nephro-CKD Panel / EDTA plasma	28	4.5	1.0-15.2	4.1

MATRIX COMPARISON

To assess whether all tested matrices behave the same way Interleukin-6 (IL-6) was measured in serum and plasma (EDTA, citrate, heparin) samples of ten apparently healthy donors (matched-paired samples).

		IL-6 [pg/ml]			% Detectable
Sample Matrix	**n**	**Mean**	**Range**	**Median**	
Serum	10	1.12	0.58-1.56	1.19	100
EDTA plasma	10	0.64	0.01-1.45	0.53	100
Citrate plasma	10	0.71	0.01-1.32	0.68	100
Heparin plasma	10	0.62	0.00-2.41	0.48	90

Measurement of Human IL-6 in Urine Samples

Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particles, assay immediately or aliquot and store at -25°C or lower. Urine samples were not normalized to creatinine values.

Sample Matrix Urine	IL-6 [pg/ml]
Donor 1. Apparently healthy	0.9
Donor 2. Apparently healthy	0.7
Donor 3. Apparently healthy	1.5
Donor 4. Apparently healthy	0.0
Donor 1. Kidney disease	122.6
Donor 2. Kidney disease	24.6
Donor 3. Kidney disease	36.6
Donor 4. Kidney disease	2.1
Donor 5. Kidney disease	13.9

Acccuracy

Data showing % recovery of recombinant IL-6 in human urine samples:

ID	IL-6 [pg/ml]		% Recovery
	Reference	+50 pg/ml	
U1	13.9	60.5	107
U2	41.7	80.7	120
U3	0.7	60.7	121
U4	1.5	58.3	115
U5	0.0	59.4	119
		Mean R [%]	**116**

Dilution linearity, parallelism

Data showing dilution linearity of endogenous IL-6 in human urine samples:

Sample ID	IL-6 [pg/ml]			R [%]	
	Ref	1+1	1+3	1+1	1+3
U1	124.8	54.8	27.0	88	86
U2	13.9	7.2	2.6	103	n.d.
U3	41.7	24.9	12.0	120	115
			Mean R [%]	**103**	**101**

Competition experiments were carried out by pre-incubating human urine samples containing endogenous IL-6 with an excess of capture antibody (AB). The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample without the pre-incubation step. Mean competition in urine samples was 100%.

Competition of endogenous signal:

Sample ID	IL-6 [pg/ml]		R [%]
	Reference	+AB	
U1	147	0.0	100
U2	16.6	0.0	100
U3	28.6	0.0	100
U4	36.1	0.0	100
U5	9.5	0.0	100
		Mean R [%]	**100**

Measurment of Human IL-6 in Cell Culture Supernatants (CCS)

Two human breast cancer cell lines MDA-MB-231, MCF-7 and a human macrophage cell line 4TL9.R were cultured in DMEM/Ham's F12 and RPMI, respectively, and supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were grown in a humidified atmosphere of 95% air and 5% CO_2 for 48 hours. Aliquots of the cell culture supernatants were removed, centrifuged to remove particles, and assayed for levels of human IL-6.

Sample Matrix CCS	IL-6 [pg/ml]
CCS - MDA-MB-231	146.6
CCS - MCF-7	18.5
CCS - 4TL9.R	0.4
Medium- DM-F-12 (with supplements)	0.0
Medium-RPMI (with supplements)	0.0

Accuracy

Recombinant IL -6 was spiked into samples by using STD7 (100pg/ml) or STD8 (200pg/ml).
Two concentration levels were generated using the following protocol:
-50 pg/ml spike: STD7 volume ratio of 1+1.
-25 pg/ml spike: STD8 volume ratio of 7+1.

CCS	ID	IL-6 [pg/ml]			R [%]	
		Reference	50	25	50	25
MDA-MB-231	CCS#1	146.6	118.2	n.d.	90	n.d.
MCF-7	CCS#2	18.5	57.9	40.7	97	98

n.d.: not determined

Dilution linearity

Dilution linearity of recombinant analyte in cell culture supernatants.
Samples were spiked with STD 7 (100pg/ml, +50pg/ml) and diluted with dilution medium (ASYBUF).

CCS	ID	IL-6 [pg/ml]				% Recovery		
		Ref	1+1	1+3	1+7	1+1	1+3	1+7
MDA-MB-231	CCS#1	118.2	53.1	27.1	13.9	90	92	94
MCF-7	CCS#2	57.9	28.0	14.1	7.1	97	97	98
					Mean R [%]	**93**	**95**	**96**

Dilution linearity of endogenous analyte in cell culture supernatants.
Performance was tested in conditioned media (48h) and diluted with dilution medium (ASYBUF).

CCS	ID	IL-6 [pg/ml]				% Recovery		
		Ref	1+1	1+3	1+7	1+1	1+3	1+7
MDA-MB-231	CCS#1	146.6	65.0	32.2	16.1	89	88	88
MCF-7	CCS#2	18.5	9.3	5.4	2.5	101	117	108
					Mean R [%]	**95**	**102**	**98**

Competition of IL-6 in cell culture supernatants

Competition experiments were carried out by pre-incubating human cell culture supernatnant samples containing endogenous IL-6 with an excess of capture antibody (AB). The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample without the pre-incubation step. Mean competition in cell culture supernatants was 98%.

Calculated according to calibration curve prepared from supplied ELISA standards (resuspended in DMEM Medium + supplements).

CCS	ID	IL-6		R [%]
		Ref	+ CAB	
MDA-MB-231	CCS#1	146.6	0.4	100
MCF-7	CCS#2	18.5	0.8	96
			Mean R [%]	**98**

REFERENCES & DOCUMENTS

Validation Literature

The assay is fully validated according to:

1. ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology"
2. EMEA/CHMP/EWP/192217/2009 Guideline on bioanalytical method validation
3. Bioanalytical Method Validation, Guidance for Industry, FDA, May 2018

Additional Documents Available Online (www.bmgrp.com)
Instructions for Use (IFU, package insert)
Material Safety Data Sheet (MSDS)

This ELISA kit was developed and manufactured by:

BIOMEDICA MEDIZINPRODUKTE GmbH
Divischgasse 4, 1210 Wien, Austria
TEL: +43/1/291 07 45
FAX: +43/1/291 07 6389
E-MAIL: info@bmgrp.com
WEB: www.bmgrp.com

COMPARISON with another human IL-6 ELISA assay

	BIOMEDICA	Another MANUFACTURER
Method	Sandwich ELISA Streptavidin-HRPO/TMB, 12x8-well detachable strips	Sandwich ELISA HRPO/TMB, 12x8-well detachable strips
Sample type	Serum, EDTA plasma, heparin plasma, citrate plasma, cell culture supernatants, urine	Serum, plasma, cell culture supernates
Sample volume	100 µl sample / well	100 µl / well 200 µl (cell culture) / well
Assay time	2 h / 1 h / 1 h / 30 min	2 h / 2 h / 30 min
Assay range	0 –200 pg/ml (0 / 3.125 / 6.25 / 12.5 / 25 / 50 / 100 / 200)	0 – 300 pg/ml
Sensitivity	LOD: 0.28 pg/ml; LLOQ: 0.78 pg/ml *(measurable concentrations in serum AND plasma samples)*	MDD: 0.7 pg/ml
Specificity	Assay recognizes recombinant and endogenous (natural) human IL-6.	Assay recognizes natural and recombinant human IL-6.
Antibodies	*Epitope-mapped antibodies* Capture antibody: recombinant IL-6 antibody specific for human IL-6 Detection antibody: polyclonal IL-6 antibody specific for human IL-6, streptavidin-HRPO-labeled	Capture antibody: monoclonal antibody specific for human IL-6 Detection antibody: polyclonal IL-6 antibody specific for human IL-6, HRPO-labeled
Standard matrix	Serum based matrix containing recombinant IL-6 *8 ready to use standards, lyophilized*	Protein based matrix containing recombinant IL-6 *1 stock standard, lyophilized*
Values of apparently healthy samples	Serum median (n=48): 1.5 pg/ml **100 % detectable** EDTA-plasma mean (n=26): 0.9 pg/ml **100 % detectable**	Serum/plasma median (n=40): 33 samples < 3.13 pg/ml 3.13 pg/ml < 7 samples < 12.5 pg/ml
Controls	**2 controls** (high and low) **included**	Not included
Validation	According to FDA/ICH/EMEA guidelines	Not indicated
Use	RUO	RUO

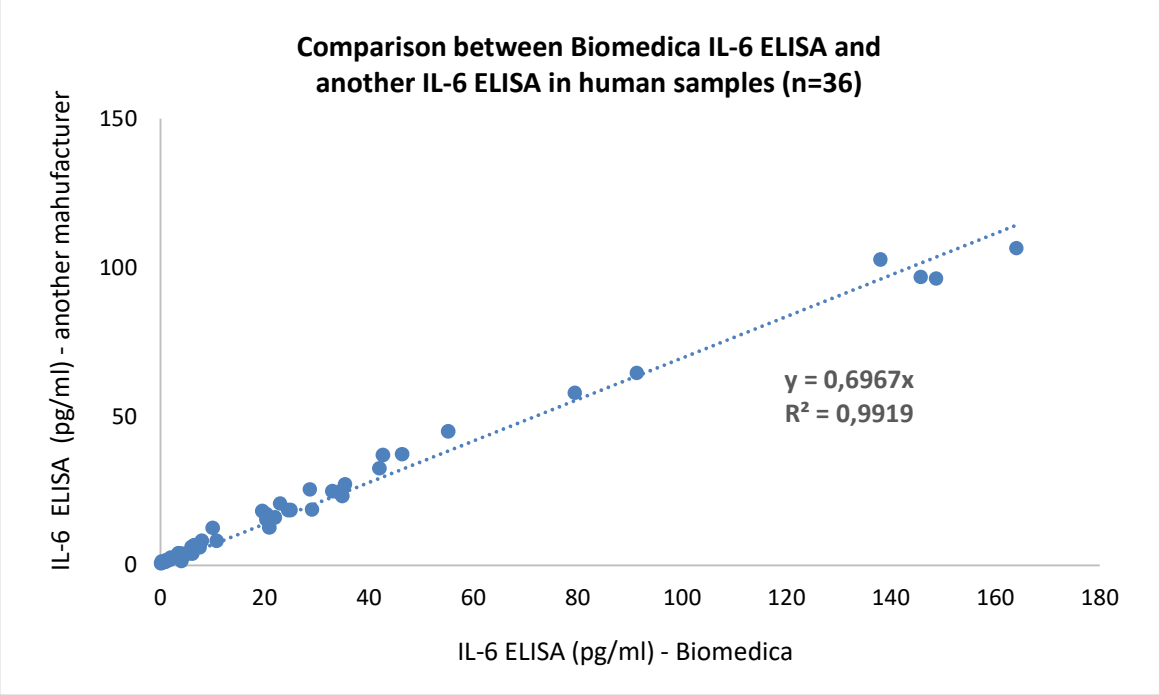
Comparison of human sample concentrations measured with different human IL-6 ELISA Assays

The Biomedica human IL-6 ELISA kit (Cat. No. BI-IL6) was compared with an ELISA kit from another manufacturer. The same panel of samples, consisting of 36 samples (healthy and diseased), were tested.

Table showing human IL-6 concentrations measured with the Biomedica human IL-6 ELISA and a human IL-6 ELISA assay from another manufacturer:

	ALL SAMPLES		
	n = 36		
Cohorts:	Sample ID	Biomedica (BI-IL6)	Other
		IL-6 [pg/ml]	IL-6 [pg/ml]
Apparently healthy cohort serum samples	AH s1	0.1	0.6
	AH s2	1.1	1.3
	AH s3	3.5	3.3
	AH s4	0.2	1.3
	AH s5	1.0	1.3
	AH s6	2.4	2.6
	AH s7	0.5	0.9
	AH s8	1.2	1.8
	AH s9	1.9	2.5
	Mean	1.31	1.75
Rheuma cohort serum samples	Rs1	46.3	37.3
	Rs2	41.9	32.6
	Rs3	55.1	45.0
	Rs4	22.9	20.7
	Rs5	20.8	12.7
	Rs6	79.4	58.0
	Mean	**44.4**	**34.4**
Cardiology panel (Aortic stensosis) serum samples	Cs1	3.4	4.1
	Cs2	6.4	6.7
	Cs3	7.9	8.2
	Cs4	42.6	37.0
	Cs5	28.6	25.5
	Mean	**17.8**	**16.3**
Unselected hospital panel Serum samples	Us1	24.4	18.5
	Us2	29.0	18.7
	Us3	24.9	18.5
	Us4	6.1	3.9
	Us5	34.8	23.3
	Us6	21.9	16.1

Unselected hospital panel EDTA plasma	Mean	**23.5**	**16.5**
	Uep1	19.5	18.2
	Uep2	10.0	12.6
	Uep3	7.5	6.1
	Uep4	4.9	3.6
	Uep5	34.2	24.4
	Uep6	34.7	25.1
	Mean	**18.5**	**15.0**
Unselected hospital panel Citrate plasma	Uc1	91.3	64.6
	Uc2	20.3	15.4
	Uc3	145.7	96.8
	Uc4	10.7	8.3
	Mean	**67.0**	**46.3**



IL-6 ELISAs Comparison – Conversion to NIBSC/WHO units

Concentrations of samples obtained in the Biomedica IL-6 ELISA and the competitor IL-6 ELISA were converted to approximate NIBSC/WHO 89/548 units according to the equation:

- NIBSC/WHO 89/548 approximate value (IU/ml) = 0.131 x obtained value (pg/ml) in competitor IL-6 ELISA
- NIBSC/WHO 89/548 approximate value (IU/ml) = 0.08 x obtained value (pg/ml) in Biomedica IL-6 ELISA

	Biomedica IL-6 ELISA (#BI-IL6)	Competitor IL-6 ELISA	ratio
Sample ID	IL-6 [pg/ml]	IL-6 [pg/ml]	
#S1	**1.95**	2.42	0.81
#S2	**2.32**	2.45	0.95
#S3	**1.99**	2.42	0.82
#S4	**0.49**	0.51	0.95
#S5	**2.79**	3.05	0.91
#S6	**1.75**	2.11	0.83
#S7	**3.70**	4.89	0.76
#S8	**3.35**	4.27	0.79
#S9	**4.41**	5.89	0.75
#S10	**1.83**	2.71	0.68
#S11	**1.66**	1.67	1.00
#S12	**6.35**	7.59	0.74
#S13	**0.27**	0.53	0.51
#S14	**0.51**	0.88	0.58
#S15	**0.63**	1.07	0.59
#S16	**3.41**	4.85	0.70
#S17	**2.29**	3.34	0.69
#H1	**2.83**	3.57	0.79
#H2	**7.45**	14.08	0.53
#H3	**0.47**	0.8	0.59
#E1	**1.56**	2.39	0.65
#E2	**0.80**	1.65	0.48
#E3	**0.60**	0.80	0.75
#E4	**0.39**	0.48	0.82
#E5	**2.74**	3.19	0.86
#E6	**2.78**	3.29	0.84
#E7	**11.03**	13.45	0.82
#C1	**7.30**	8.46	0.86
#C2	**1.62**	2.02	0.80
#C3	**11.65**	12.69	0.92
#C4	**0.86**	1.08	0.79
#U1	**9.81**	12.2	0.80
#U2	**1.97**	2.99	0.66
#U3	**0.32**	0.52	0.61
#CCM1	**13.12**	13.95	0.94
#CCM2	1.63	2.26	0.72

Abbreviations: S (serum), H (heparin plasma), E (EDTA plasma), C (citrate plasma), U (urine), CCM (cell culture supernatants)

Conclusion:
Concentration values obtained in the Biomedica IL-6 ELISA converted to IU/ml using NIBSC/WHO 89/548 measure very similar to the competitor IL-6 ELISA in all matrices. Correlation of both assays is excellent – Pearson correlation coefficient $R = 0.978$, $p < 0.00001$.

Comparison of cell culture supernatant sample concentrations in human breast cancer cell lines measured with different human IL-6 ELISA Assays

The Biomedica IL-6 ELISA (#BI-IL6) was compared with an ELISA kit from another manufacturer.

Cell Culture Supernatants (CCS)		
n = 2		
Sample ID	Biomedica (# BI-IL6)	Other ELISA
	IL-6 [pg/ml]	IL-6 [pg/ml]
CCS - MDA-231	164.0	106.5
CCS - MCF-7	20.3	17.3