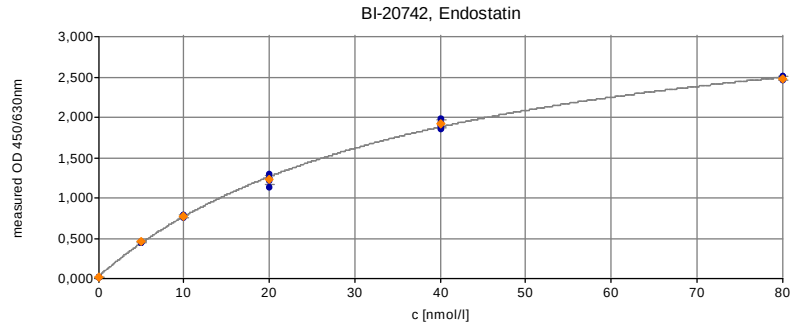
ENDOSTATIN ELISA, Cat.No. BI-20742

For the quantitative determination of Endostatin in human samples

ASSAY CHARACTERISTICS

Method	Sandwich ELISA, 96-well strip plate, HRP/TMB
Sample type	Serum, plasma (EDTA, citrate, heparin), urine protocol available.
Standard range	0-80 nmol/l (= 0-1600 ng/ml)
Conversion factor	1 ng/ml = 0.05 nmol/l (MW = 20 kDa)
Sample volume	5 µl neat sample (before dilution)
Detection limit	0.2 nmol/l (0 nmol/l + 3 SD)
Incubation time, temp.	3 h / 1 h / 30 min, room temperature (18-26°C)
Cross reactivity	Human only, no cross-reactivity with other species

Typical standard curve:



Values from apparently healthy individuals:

Sample type	Serum (n=59)	Citrate plasma (n=30)
Median (nmol/l)	5.1 (3.3 – 7.8)	4.7 (3.3 – 7.9)

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

Values from unselected hospital panel 1:

(EDTA plasma, citrate plasma, and heparin plasma samples derive from different donors)

Sample type	EDTA plasma (n=20)	Citrate plasma (n=20)	Heparin plasma (n=20)
Median (nmol/l)	38.7	31.3	35.2
Min (nmol/l)	28.6	23.0	27.0
Max (nmol/l)	56.8	64.0	62.4

Values from unselected hospital panel 2:

(Serum, EDTA plasma, and heparin plasma samples derive from different donors)

Sample type	Serum (n=40)	EDTA plasma (n=40)	Heparin plasma (n=40)
Median (nmol/l)	21.5	20.7	21.5
Min (nmol/l)	8.7	9.5	10.9
Max (nmol/l)	38.1	29.7	30.1

PERFORMANCE CHARACTERISTICS

Spike/Recovery of recombinant Endostatin:

Recovery of spiked human samples was tested by adding different concentrations of recombinant Endostatin (5, 20, 40 nmol/l) to human serum and plasma samples.

The mean recovery of recombinant Endostatin in serum and citrate plasma is 95% and 91%, respectively.

The mean recovery of recombinant Endostatin in heparin plasma and EDTA plasma is 96% and 92%, respectively.

Data show spike/recovery of human serum and plasma samples:

Sample	Mean recovery (%)	Range (%)
Serum (n=5)	95	72 – 127
Citrate plasma (n=4)	91	78 – 119
Heparin plasma (n=4)	96	68 – 139
EDTA plasma (n=4)	92	64 – 143

Dilution linearity of endogenous Endostatin:

Dilution linearity of endogenous Endostatin in human samples with ASYBUF (assay buffer):

Dilution		Serum (n=3)	Citrate plasma (n=3)	EDTA plasma (n=3)	Heparin plasma (n=3)
1:30	Mean recovery (%)	119	108	127	116
	Range (%)	107 – 130	102 – 114	117 – 132	112 – 123
1:60	Mean recovery (%)	120	100	138	114
	Range (%)	99 – 129	91 – 114	120 – 160	111 – 120
1:120	Mean recovery (%)	116	95	141	120
	Range (%)	101 – 127	90 – 104	120 – 163	119 – 121

Intra-assay precision & inter-assay precision:

Intra-assay: 2 samples of known concentrations were tested 5 times within 1 kit lot by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 16 times within 2 different kit lots and by 3 different operators.

Intra-assay (n=5)	Sample 1	Sample 2	Inter-assay (n=16)	Sample 1	Sample 2
Mean (nmol/l)	5.2	41.6	Mean (nmol/l)	5.0	40.5
SD (nmol/l)	0.09	2.36	SD (nmol/l)	0.21	2.16
CV (%)	2	6	CV (%)	4	5

SAMPLE CHARACTERISTICS

Effect of sample matrix:

The CV of endogenous Endostatin in 4 samples of different matrices (serum, EDTA plasma, heparin plasma, citrate plasma) within 1 donor is 4-11%.

Experiment: 4 samples of different matrices (serum, EDTA plasma, heparin plasma, citrate plasma) from 7 individual donors were tested for endogenous Endostatin levels.

Data show endogenous Endostatin levels in different matrices from individual donors:

Sample ID	Serum (nmol/l)	Heparin plasma (nmol/l)	Citrate plasma (nmol/l)	EDTA plasma (nmol/l)	CV (%)
#1	5.4	5.1	4.9	4.8	5
#2	4.3	4.2	3.8	3.9	6
#3	5.7	5.9	4.7	4.9	11
#4	7.1	6.6	6.3	7.1	6
#5	6.2	5.7	5.6	5.6	5
#6	7.1	6.7	6.2	7.0	6
#7	7.0	6.7	6.6	6.3	4
				Mean CV (%)	6

Stability of samples:

We recommend performing serum or plasma separation by centrifugation as soon as possible, e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C). If this is not possible store the samples at 4°C (2-8°C) prior to centrifugation (up to 1 day).
The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C, for long time storage at -80°C. All samples should undergo only 4 freeze-thaw cycles.

Whole blood stability:
Freshly collected samples (n=12) were measured immediately and after 2 h, 4 h, and overnight storage at room temperature (18-26°C). The recovery of stored samples at room temperature (18-26°C) for up to 1 day showed a mean CV of 5%. Thus samples are stable at least for 24 h at room temperature (18-26°C).

Stability of diluted samples:
Samples are stable when diluted 1+100 in ASYBUF (assay buffer) and left at 4°C (2-8°C) over night. The recovery of diluted samples showed a mean CV of 3%. Thus dilution of samples can be prepared 1 day before analysis.

Effect of freezing/thawing:

The mean CV of human serum samples after a 4 times freeze-thaw cycle is 5%. Thus samples can be frozen at least 4 times.

Experiment: Fresh samples were thawed the first time and these values count as "1x F/T". Then they were frozen up to 4 times by storing them for 60 min at -25°C. The thawing process was at room temperature (18-26°C) for 60 min.

Data show calculated concentrations, the CV, and the recovery of samples and aliquots which were stressed by 4 freeze-thaw cycles:

sample ID	c (nmol/l)				Mean (nmol/l)	CV (%)	R (%)
	1x F/T	2x F/T	3x F/T	4x F/T			
#1	5.2	5.3	4.9	5.1	5.1	3	99
#2	9.3	9.2	9.3	10.7	9.6	8	115
#3	8.2	8.4	8.0	8.4	8.2	3	102
#4	7.5	7.4	7.4	8.2	7.6	5	110

Applicability of urine samples:

The assay is not validated for urine as a sample matrix.

The Biomedica Endostatin ELISA assay can be applied for the measurement of urine samples for R&D purposes.

A special "urine protocol" for the measurement of Endostatin in urine samples has been adapted and is separately available.

Data show endogenous Endostatin levels in human urine samples:

	Apparently healthy (n=41)	Renal disease (n=22)
Median (pmol/l)	45	182
Min (pmol/l)	11	13
Max (pmol/l)	179	806

Spike/recovery of recombinant human Endostatin in human urine samples:

5 human urine samples were spiked with recombinant human Endostatin (c=100 pmol/l). The mean recovery of recombinant Endostatin in urine samples is 71%.

Dilution linearity of endogenous Endostatin in urine samples:

Dilution		Urine (n=5)
1:3	Mean recovery (%)	120
	Range (%)	97 - 138

Competition data of human urine sample spiked with recombinant human Endostatin using the unlabeled capturing antibody:

4 human urine samples were spiked with human recombinant Endostatin (c=100 pmol/l). The signal in all samples could be diminished by 100% after addition of the unlabeled anti-human Endostatin capturing antibody.