

## Big Endothelin 1 ELISA

for the quantitative determination of human  
Big Endothelin 1 in serum, EDTA plasma, heparin plasma, and citrate plasma  
Cat. No. BI-20082H . 12 x 8 tests

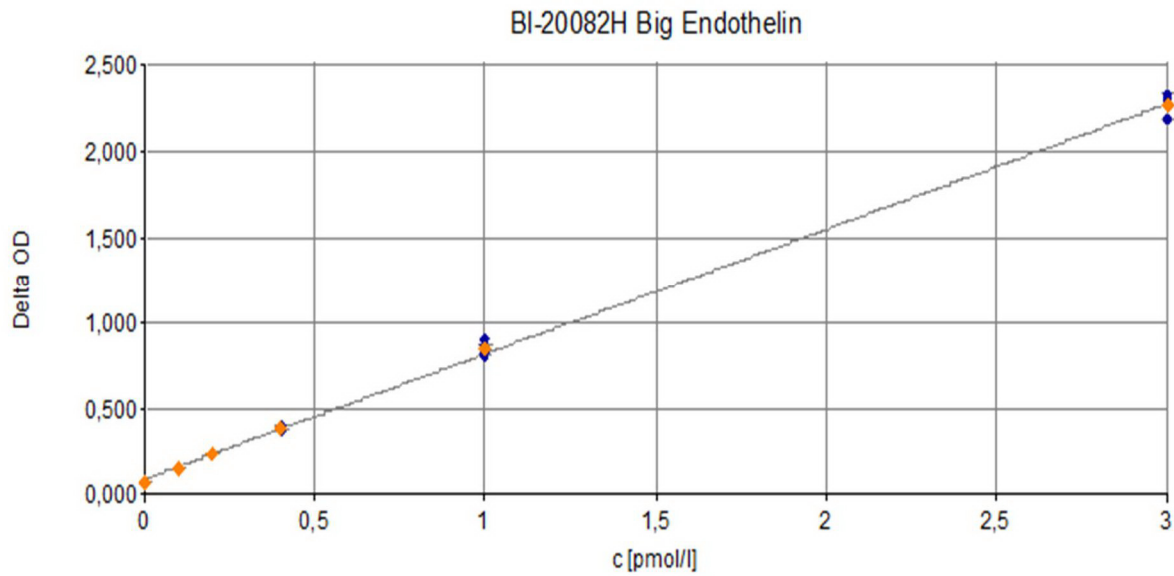
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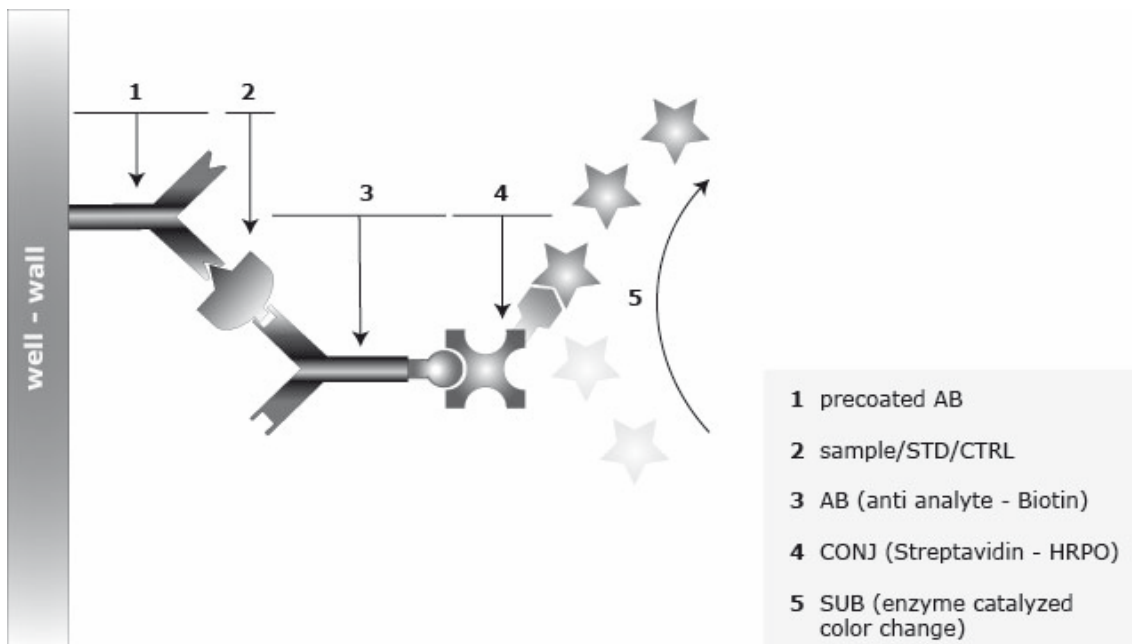
## ASSAY CHARACTERISTICS Summary

<b>Method</b>	Sandwich ELISA, HRP/TMB, 12x8-well strips		
<b>Sample type</b>	Serum, EDTA plasma, heparin plasma, and citrate plasma		
<b>Standard range</b>	0 to 3 pmol/l (6 standards and 1 control in a human serum matrix). Standards: 0/0.1/0.2/0.4/1/3 pmol/l		
<b>Conversion factor</b>	1 pg/ml = 0.2335 fmol/ml (MW: 4.283 kDa)		
<b>Sample volume</b>	50 µl / well		
<b>Incubation time, temp.</b>	4 h / 1 h / 30 min, room temperature		
<b>Sensitivity</b>	LOD: (0 pmol/l + 3 SD): 0.02 pmol/l; LLOQ: 0.03 pmol/l		
<b>Specificity</b>	This assay recognizes endogenous and recombinant human Big ET-1 (1-38).		
<b>Cross-reactivity</b>	ET1/2/3 (1-21): <1%, ET2 (1-37): <1%, ET1/2 (1-38): <1%, porcine BigET (1-39): 21%, BigET1/2 (22-38) : <1%, BigET2 (22-37) : <1%, rat BigET1 (1-39): 10%, Sarafotoxin: <1%		
<b>Precision</b>	Intra-assay (n=5) ≤ 5%, Inter-assay (n=10) ≤ 4%		
<b>Spike/Recovery</b>	<u>Average % recovery spiked with 1 pmol/l and 2 pmol/l</u>	Serum (n=14): 100; 105 EDTA plasma (n=3): 100; 101 Heparin plasma (n=3): 97; 102 Citrate plasma (n=3): 98; 105	
<b>Dilution linearity of endogenous Big ET-1</b>	Average % of expected dilution:	1+1	1+3
	Serum (n=8)	90	96
	EDTA plasma (n=4)	110	104
<b>Values of apparently healthy individuals</b>	Median serum (n=41) = 0.09 pmol/l It is recommended to establish the normal range for each laboratory.		

## TYPICAL STANDARD CURVE



## PRINCIPLE OF THE ASSAY



CAB coating antibody: polyclonal sheep IgG  
DAB detection antibody: monoclonal mouse IgG  
STD standard: recombinant human Big ET (1-38) in human serum

## SAMPLE VALUES

### Big ET-1 levels in an apparently healthy cohort and unselected hospital panels

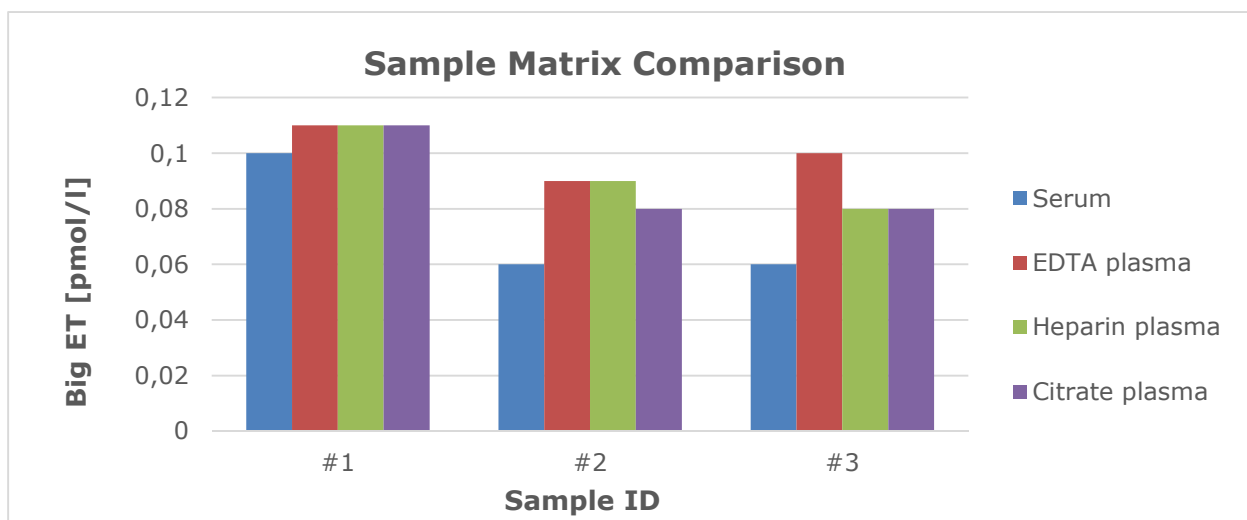
	Big ET-1 [pmol/l]		
	App. healthy (serum, n=41)	Cardio panel (serum, n=30)	Dialysis panel (serum, n=36)
Mean	0.22	0.8	1.1
<b>Median</b>	<b>0.09</b>	<b>0.6</b>	<b>0.9</b>
Min	0.01	0.2	0.4
Max	1.58	2.1	3.0

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

## MATRIX COMPARISON

### Correlation of serum and plasma samples from apparently healthy individuals

Sample ID	Big ET [pmol/l]				Mean [pmol/l]	CV [%]
	Serum	EDTA plasma	Heparin plasma	Citrate plasma		
#1	0.1	0.11	0.11	0.11	0.11	7
#2	0.06	0.09	0.09	0.08	0.08	17
#3	0.06	0.1	0.08	0.08	0.08	23



## ASSAY PERFORMANCE CHARACTERISTICS

### RECOVERY

Summary of data showing mean recovery of recombinant Big ET:

Matrix	Mean S/R [% ]	
	+1 pmol/l	+2 pmol/l
Serum (n=14)	100	105
EDTA plasma (n=3)	100	101
Heparin plasma (n=3)	97	102
Citrate plasma (n=3)	98	105

#### Experiments:

Recovery of spiked samples was tested by adding 2 concentrations of human recombinant Big ET (1 + 2 pmol/l) to different human sample matrices.

Data showing spike/recovery of human serum samples:

Sample ID	Spike Big ET [pmol/l]			S/R [%]	
	0	1	2	1	2
#S1	0.65	1.6	2.7	97	102
#S2	0.8	2.1	2.9	117	104
#S3	0.02	1.3	2.4	127	119
#S4	0.3	1.5	2.4	115	104
#S5	0	1.1	2.5	110	125
#S6	0	0.9	2.2	90	110
#S7	0	1.0	2.3	100	115
#S8	0	0.8	1.7	80	85
#S9	0	0.8	1.9	80	95
#S10	0	1.1	1.9	110	95
#S11	0	1.0	2.1	100	105
#S12	0.1	1.1	2.3	100	110
#S13	0.06	0.9	1.8	85	87
#S14	0.06	0.9	2.3	85	112
			<b>Mean R [%]</b>	<b>100</b>	<b>105</b>

Data showing spike/recovery of human EDTA plasma samples:

Sample ID	Spike Big ET [pmol/l]			S/R [%]	
	0	1	2	1	2
#E1	0.11	1.2	2.4	108	114
#E2	0.09	1.1	2.1	101	100
#E3	0.1	1.0	2.0	91	95
			<b>Mean R [%]</b>	<b>100</b>	<b>101</b>

Data showing spike/recovery of human heparin plasma samples:

Sample ID	Spike Big ET [pmol/l]			S/R [%]	
	0	1	2	1	2
#H1	0.11	1.2	2.3	108	109
#H2	0.09	1.0	2.0	92	96
#H3	0.08	1.0	2.1	93	101
<b>Mean R [%]</b>				<b>97</b>	<b>102</b>

Data showing spike/recovery of human citrate plasma samples:

Sample ID	Spike Big ET [pmol/l]			S/R [%]	
	0	1	2	1	2
#C1	0.11	1.1	2.4	99	114
#C2	0.08	1.2	2.2	111	106
#C3	0.08	0.9	2.0	83	96
<b>Mean R [%]</b>				<b>98</b>	<b>105</b>

## LINEARITY

### Dilution linearity of samples containing endogenous and recombinant Big ET

Matrix	Mean R of dilution steps [%]	
	1+1	1+3
Serum (n=8)	90	96
EDTA plasma (n=4)	110	104

► We recommend diluting high measuring samples (outside of the calibration range) in STD1 (standard matrix, 0 pmol/l).

### Experiment:

Dilution linearity was assessed by serially diluting samples containing endogenous Big ET with STD1 (standard matrix, 0 pmol/l, provided in the kit).

Data showing the dilution of endogenous Big ET in serum samples:

Sample ID	Big ET [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#S1	1.36	0.42	0.24	62	71
#S2	0.24	0.13	0.08	105	126
#S3	0.48	0.25	0.13	103	110
#S4	1.46	0.64	0.33	87	92
#S5	0.78	0.37	0.20	94	102
#S6	1.43	0.56	0.31	79	87
#S7	3.11	1.47	0.59	95	76
#S8	2.67	1.28	0.69	96	104
<b>Mean R [%]</b>				<b>90</b>	<b>96</b>

Data showing the dilution of recombinant Big ET (spike 2pM) in EDTA plasma samples

Sample ID	Big ET [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#E1	2.06	1.30	0.64	127	124
#E2	2.58	1.38	0.63	107	97
#E3	2.30	1.23	0.61	107	106
#E4	2.64	1.31	0.60	99	91
<b>Mean R [%]</b>				<b>110</b>	<b>104</b>

### Recommendations for sample dilution

High measuring samples outside of the calibration range of the curve should be diluted in STD1 (provided in the kit). The kit standard matrix is a human serum containing 0 pmol/l Big ET concentrations.

### PRECISION

#### Intra-assay precision & Inter-assay precision

Intra-assay (n=5) ≤ 5%, Inter-assay (n=10) ≤ 4%

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 10 times within 3 different kit lots by different operators.

<b>Intra-assay (n=5)</b>	<b>Sample 1</b>	<b>Sample 2</b>	<b>Inter-assay (n=10)</b>	<b>Sample 1</b>	<b>Sample 2</b>
Mean (pmol/l)	0.20	1.00	Mean (pmol/l)	0.20	1.00
SD (pmol/l)	0.003	0.048	SD (pmol/l)	0.009	0.041
CV (%)	2	5	CV (%)	4	4

### SENSITIVITY

#### Limit of detection (LOD)

The LOD is defined as the mean value of the back calculated concentration plus three times the standard deviation. The LOD of the Big ET ELISA is **0.02 pmol/l**.

#### Lower limit of quantification (LLOQ)

The lower limit of quantification is defined as the accuracy of the back calculated concentrations and shall not exceed ±25% (acc. to ICH [Ref. 1]).

For the Big ET ELISA the LLOQ is **0.03 pmol/l**.

## SAMPLE STABILITY

**Big Endothelin-1 (BigET)** is a peptide of 38 amino acids and is the precursor of Endothelin-1 (ET), represented by amino acids 1-21 (<http://www.uniprot.org/uniprot/P05305>). ET is a potent vasoconstrictor and is produced by vascular endothelial cells. Accordingly it has a wide tissue distribution (<http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.511899>).

The cleavage of BigET by Endothelin Converting Enzyme (ECE) leads to ET and to a C-terminal fragment. Both BigET and ET are strong independent predictors of survival in patients with congestive heart failure, and identify a population with very high risk mortality. The half-life of ET (1-21) in plasma is less than one minute, whereas clearance of BigET is much slower. BigET can therefore be determined more easily.

### Sample preparation

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).

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.

### Freeze/thaw of serum samples containing endogenous Big ET

#### **Serum samples can undergo 4 freeze-thaw cycles.**

The mean recovery of sample concentrations stressed by 4 F/T cycles is 109%.

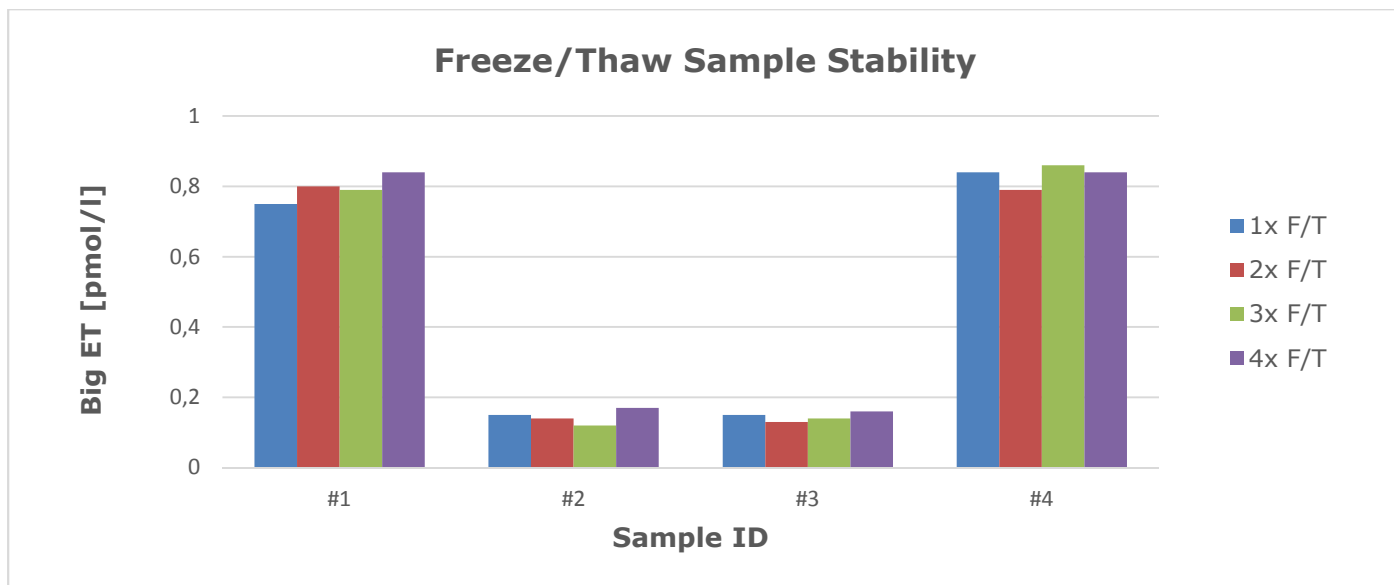
The mean CV of sample concentrations (not stressed and stressed up to 4 times by freeze-thaw cycles) is 8%.

Serum samples are stable for at least 4 freeze-thaw cycles.

Data showing Big ET concentrations of samples after freeze-thaw cycles:

Sample ID	Big ET [pmol/l]				Mean F/T	CV [%]	R [%] 4 F/T vs 1x F/T
	1x F/T	2x F/T	3x F/T	4x F/T			
S1	0.75	0.80	0.79	0.84	0.80	4	111
S2	0.15	0.14	0.12	0.17	0.14	16	120
S3	0.15	0.13	0.14	0.16	0.15	8	103
S4	0.84	0.79	0.86	0.84	0.83	3	100
					<b>Mean [%]</b>	<b>8</b>	<b>109</b>





## SPECIFICITY

This assay recognizes endogenous (natural) and recombinant human Big ET.

The factors listed below were assayed for cross-reactivity:

Human ET1/2/3 (1-21): <1%, human ET2 (1-37): <1%, human ET1/2 (1-38): <1%, porcine BigET (1-39): 21%, human BigET1/2 (22-38) : <1%, human BigET2 (22-37) : <1%, rat BigET1 (1-39): 10%, Sarafotoxin: <1%

## CALIBRATION

This immunoassay is calibrated against recombinant human Big ET (1-38).

## Validation

**The assay is fully validated according to ICH Q2 (R1), Ref. [1].**

[1] CPMP/ICH/381/95 ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology” including:

ICH Q2A “Text on Validation of Analytical Procedures”

ICH Q2B “Validation of Analytical Procedures: Methodology”

*Date: March 2015*