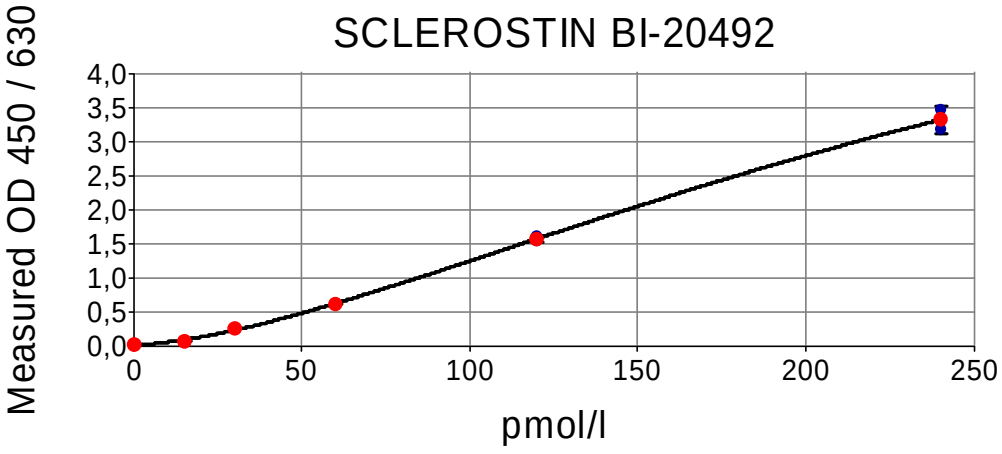
Sclerostin ELISA (Cat.No. BI-20492)

For the Determination of Sclerostin in Human Samples

ASSAY CHARACTERISTICS

Method	Sandwich ELISA, HRP/TMB
Sample type	Serum, plasma (Citrate, EDTA, Heparin)
Standard range	6 standards diluted in a serum matrix ranging from 0-240 pmol/l (0 / 15 / 30 / 60 / 120 / 240 pmol/l) and 1 serum based control.
Conversion factor	1 pg/ml = 0.044 pmol/l (MW: 22.5 kDa)
Sample volume	20 µl
Detection limit	3.2 pmol/l (0 pmol/l + 3 SD)
LLOQ	<7.5 pmol/l
Serum values of apparently healthy individuals	Median 24.14 pmol/l (n=411) ***This value lies between calibration point 2 and 3 of the standard curve.***
Incubation time, temp.	18-24 h / 1 h / 30 min, room temperature
Cross reactivity	The assay does not cross-react with rat or mouse samples. The antibodies utilized in the Biomedica Sclerostin ELISA show no cross-reactivity with Wise (SOSTDC1) or Noggin.

Typical Standard Curve:



Screening of 411 human donor sera from apparently healthy individuals:

	Sclerostin [pmol/l]
Mean value	27.54
Median	**24.14**
Percentil 98%	64.59
Percentil 95%	52.02
Percentil 5%	10.78
Percentil 2%	7.52
Standard Deviation SD	14.23
Number of sera	411

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

Screening of 15 human donor sera from unselected hospital panel:

	Sclerostin [pmol/l]
Number of sera	15
Mean value	56
Median	**57**
Min	6
Max	135
Percentil 95%	118
Percentil 5%	9

PERFORMANCE CHARACTERISTICS:

Spike Recovery:

The mean recovery of recombinant Sclerostin in human serum samples (n=6) is 94%.

Experiment: Recovery of spiked human serum samples was tested by adding 2 defined concentrations of recombinant Sclerostin in 6 different human serum samples (sera were spiked with standards provided with the kit: STD 5 and STD 6 containing 120 and 240 pmol/l human recombinant Sclerostin, respectively). Sera were spiked directly into the well (e.g. 10 µl serum + 10 µl Standard material STD 5 or STD 6).

Data showing recovery of human recombinant Sclerostin in human serum samples:

Sample ID	unspiked	spike 1+1 with STD5 [pmol/l]	S/R [%]	spike 1+1 with STD6 [pmol/l]	S/R [%]
Serum #1	25.1	73.4	101	126.7	95
Serum #2	32.8	72.2	93	133.1	97
Serum #3	29.3	72.4	96	115.3	84
Serum #4	33.2	76.1	99	123.4	89
Serum #5	28.5	70.0	93	119.0	87
Serum #6	31.3	75.1	99	117.3	85
Mean S/R [%]			**97**		**90**

Dilution Linearity:

Endogenous Sclerostin:
The mean dilution linearity of endogenous Sclerostin in human serum samples (n=4) is 110% for dilution factors 1+1, 1+3, 1+7 in assay buffer.

Experiment: Dilution linearity was assessed by a serial dilution of 4 human serum samples from different donors containing elevated Sclerostin levels with assay buffer.

Dilution linearity of endogenous Sclerostin	Summary
Mean for 1+1 dilution	110%
Mean for 1+3 dilution	113%
Mean for 1+7 dilution	106%

Data showing dilution of human serum samples containing endogenous Sclerostin:

Sample ID	dilution	c [pmol/l]	dil lin R [%]
Serum #1	undiluted	**176**	
	1+1	106	121
	1+3	57	130
	1+7	31	139
Serum #2	undiluted	**75**	
	1+1	36	96
	1+3	19	99
	1+7	7	72
Serum #3	undiluted	**50**	
	1+1	27	108
	1+3	13	102
	1+7	6	93
Serum #4	undiluted	**242**	
	1+1	142	117
	1+3	74	122
	1+7	36	119

Recombinant Sclerostin:
The mean dilution linearity of recombinant Sclerostin in human serum samples (n=3) is 98% for dilution factors 1+1, 1+3 in assay buffer.

Experiment: Dilution linearity was assessed by a serial dilution of 3 serum samples spiked with recombinant Sclerostin with assay buffer.

Dilution linearity of recombinant Sclerostin	Summary
Mean for 1+1 dilution	103%
Mean for 1+3 dilution	93%

Data showing dilution of human serum samples containing recombinant Sclerostin:

			c [pmol/l]			
sample ID	spike [pmol/l]	dilution factor	unspiked sample	spiked sample	diluted sample	R [%]
Serum #1	100		10.0	**99.5**		
		1+1			51.6	104
		1+3			22.5	90
Serum #2	100		10.2	**96.1**		
		1+1			51.1	106
		1+3			24.0	100
Serum #3	100		7.9	**85.8**		
		1+1			42.7	100
		1+3			19.0	89

Intra-assay precision & Inter-assay precision
The intra-assay precision of the Sclerostin ELISA is ≤7%.
The inter-assay precision of the Sclerostin ELISA is ≤10%.

Experiment:
Intra-assay: 2 samples of known concentrations were tested 8 times by one operator within one kit lot.
Inter-assay: 2 samples of known concentrations were tested 6 times within 3 different assay lots by two different operators.

Data showing intra-assay and inter-assay precision:

Intra-assay (n=8)	Sample 1	Sample 2	Inter-assay (n=6)	Sample 1	Sample 2
Mean [pmol/l]	33.6	118.8	Mean [pmol/l]	30.5	120.2
SD [pmol/l]	2.37	5.36	SD [pmol/l]	3.19	3.67
CV [%]	7	5	CV [%]	10	3

Detection limit
The detection limit is defined as the mean value of the back calculated concentration plus three times the standard deviation. The **detection limit** of the Sclerostin ELISA is **3.2 pmol/l**.

The 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 Sclerostin ELISA the **LLOQ is <7.5pmol/l**.

SAMPLE CHARACTERISTICS:

Effect of sample matrix:

Experiment 1:
Measurement of Sclerostin in 4 different sample matrices from 8 samples of apparently healthy individuals showed a mean CV of 13%.

Data showing the effect of the sample matrix:

Matrix	Serum	Heparin plasma	EDTA plasma	Citrate plasma	Mean [pmol/l]	SD [pmol/l]	CV [%]
Sample ID	c [pmol/l]						
#1	26.4	25.4	20.,0	23.2	23.8	2.8	12
#2	22.4	19.8	16.3	19.6	19.5	2.5	13
#3	26.9	22.9	18.8	23.6	23.0	3.3	15
#4	17.1	14.6	12.1	14.2	14.5	2.0	14
#5	28.3	26.8	22.7	26.4	26.1	2.4	9
#6	28.2	31.5	21.9	29.1	27.7	4.1	15
#7	27.9	26.0	22.0	25.4	25.3	2.5	10
#8	18.5	16.7	11.6	13.5	15.1	3.1	20
						Mean CV [%]	**13**

Experiment 2:
Measurement of Sclerostin in 3 different sample matrices from 6 samples of individuals with an elevated Sclerostin level showed a mean CV of 6%.

Data showing the effect of the sample matrix:

Plasma	EDTA	Heparin	Citrate	Mean c [pmol/l]	CV [%]
Sample ID	c [pmol/l]				
#1	129.2	129.6	121.1	126.6	4
#2	23.0	20.4	19.4	20.9	9
#3	54.7	55.0	50.0	53.2	5
#4	28.1	28.1	24.1	26.8	9
#5	104.4	110.3	102.5	105.7	4
#6	103.9	110. 7	107.0	107.2	3
				Mean CV [%]	**6**

Stability of samples:

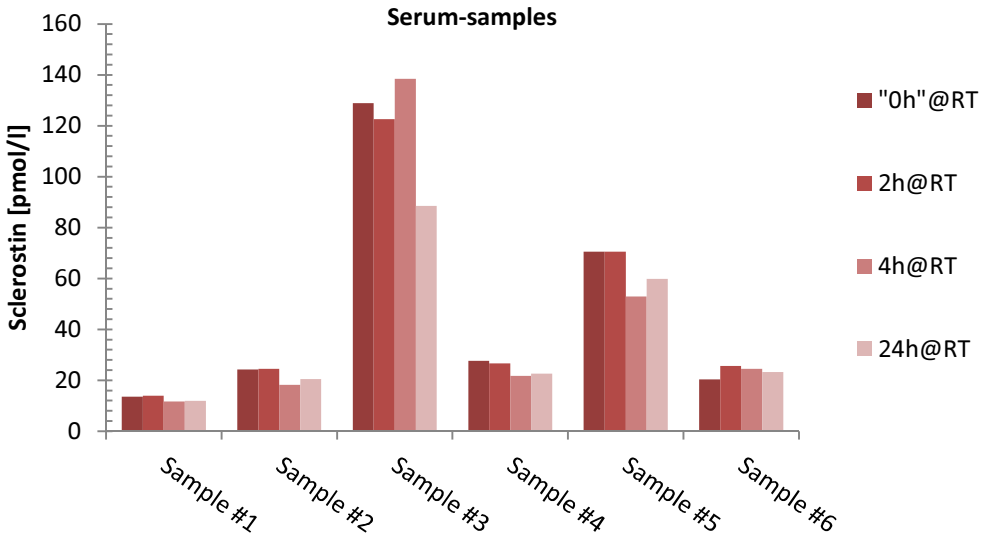
We recommend performing serum or plasma separation by centrifugation as soon as possible (e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C)). If this is not possible store the samples at 4°C (2-8°C) prior to centrifugation (up to one day).

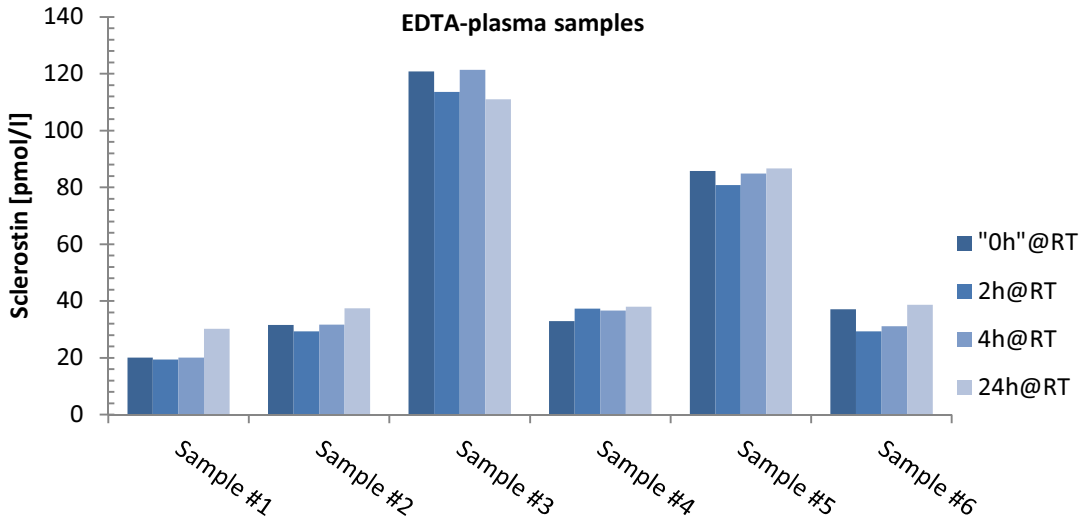
The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C, for long time storage at -80°C. All samples should undergo only 4 freeze-thaw cycles. Serum samples can be stored for ≥ 2 years at - 80°C.

Whole blood stability:

Experiment:

Stability of Sclerostin in whole blood was tested in "serum tubes" (BD Vacutainer CAT) and "EDTA tubes" (BD Vacutainer K2E), directly after collection and after 2h, 4h and 24h.





Figures: Stability of Sclerostin in serum samples (red) and EDTA-plasma samples (blue). Samples were freshly collected and measured in different time intervals at room temperature.

Conclusion: The results show that Sclerostin is stable in both serum samples and EDTA-plasma samples. Serum samples can be stored for at least 4h at room temperature. EDTA-plasma samples can be stored for at least 24h at room temperature.

Freeze/thaw of serum samples containing endogenous Sclerostin:

Serum samples can undergo 4 freeze/thaw cycles.
The mean CV of 4 human serum samples containing different levels of endogenous Sclerostin after 4 freeze/thaw cycles is 3%.

Cross reactivity:

The assay does not detect Noggin.
The assay does not detect Wise (SOSTDC1).

Species cross reactivity:

Rat, mouse: The assay does not detect rat or mouse Sclerostin.

Primates: The sequence homology of human Sclerostin to various primate species is >95%. It is likely that the assay can be used for these species. Internal validations have not been carried out. However, data from other laboratories show positive results.

Sequences from top to bottom:
Human Sclerostin sequence, Olive baboon (Papio anubis), Macaca mulatta (Rhesus macaque), Macaca fascicularis (Cynomologus monkey).

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1    MQLPLALCLVCLLVHTAFRVVEGQGWQAFKNDATEIIPELGEYPEPPPELENNKTMNRAE   60  Q9BQB4   SOST_HUMAN
1    MQLPLALCLVCLLVHAAFRVVEGQGWQAFKNDATEIIPELGEYPEPPPELENNKTMNRAE   60  gi|402900453|ref|XP_003913189.1|
1    MQLPLALCLVCLLVHAAFRVVEGQGWQAFKNDATEIIPELGEYPEPPPELENNKTMNRAE   60  F6WYL4   F6WYL4_MACMU
1    MQLPLALCLVCLLVHAAFRVVEGQGWQAFKNDATEIIPELGEYPEPPPELENNKTMNRAE   60  G7PUY1   G7PUY1_MACFA
     **************:**************************************************

61   NGGRPPHHPFETKDVSEYSCRELHFTRYVTDGPCRSAKPVTELVCSGQCGPARLLPNAIG  120  Q9BQB4   SOST_HUMAN
61   NGGRPPHHPFETKDVSEYSCRELHFTRYVTDGQCRSAKPVTELVCSGQCGPARLLPNAIG  120  gi|402900453|ref|XP_003913189.1|
61   NGGRPPHHPFETKDVSEYSCRELHFTRYVTDGQCRSAKPVTELVCSGQCGPARLLPNAIG  120  F6WYL4   F6WYL4_MACMU
61   NGGRPPHHPFETKDVSEYSCRELHFTRYVTDGQCRSAKPVTELVCSGQCGPARLLPNAXX  120  G7PUY1   G7PUY1_MACFA
     ******************************** ************************

121  RGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASCKCKRLTRFHNQSELK  180  Q9BQB4   SOST_HUMAN
121  RGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGAAPRARKVRLVASCKCKRLTRFHNQSELK  180  gi|402900453|ref|XP_003913189.1|
121  RGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGAAPRARKVRLVASCKCKRLTRFHNQSELK  180  F6WYL4   F6WYL4_MACMU
121  RGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGAAPRARKVRLVASCKCKRLTRFHNQSELK  180  G7PUY1   G7PUY1_MACFA
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181  DFGTEAARPQKGRKPRPRARSAKANQAELENAY  213  Q9BQB4   SOST_HUMAN
181  DFGPEAARPQKGRKPRPRARGAKANQAELENAY  213  gi|402900453|ref|XP_003913189.1|
181  DFGPEAARPQKGRKPRPRARGAKANQAELENAY  213  F6WYL4   F6WYL4_MACMU
181  DFGPEAARPQKGRKPRPRARGAKANQAELENAY  213  G7PUY1   G7PUY1_MACFA
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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: September 2013