

## **bioactive Sclerostin ELISA**

for the quantitative determination of human bioactive Sclerostin  
in serum, EDTA plasma, and citrate plasma  
Cat. No. BI-20472 . 12 x 8 tests

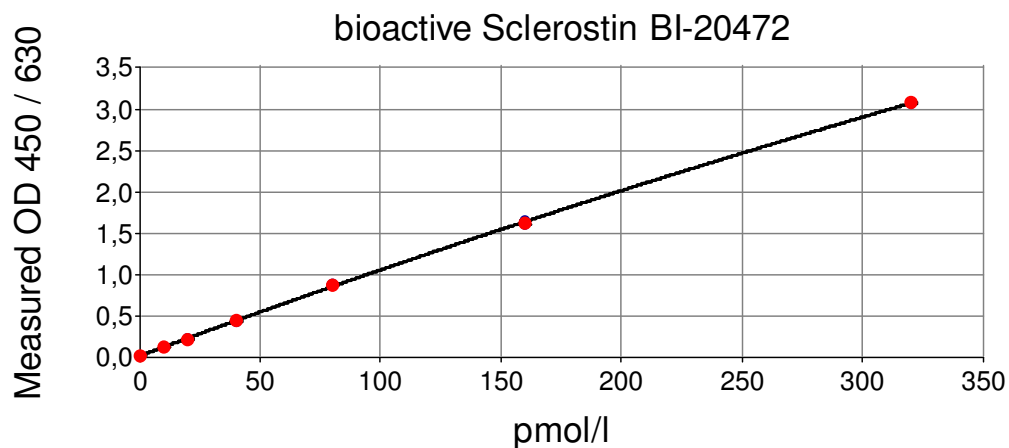
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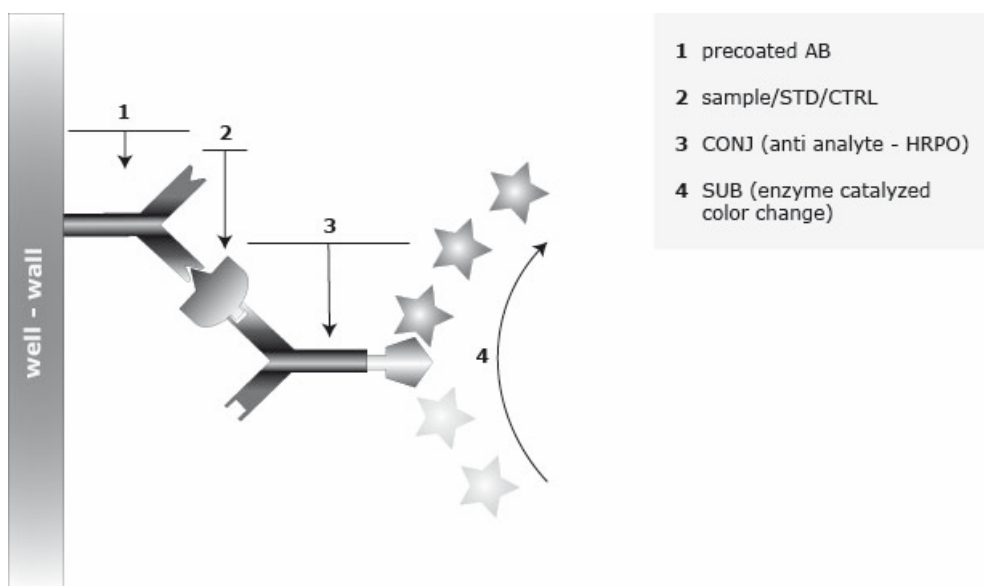
### ASSAY CHARACTERISTICS Summary

|  |   |   |            |            |
|--|---|---|------------|------------|
| <b>Method</b>  | Sandwich ELISA, HRP/TMB, 12x8-well strips   |   |            |            |
| <b>Sample type</b>   | Serum, EDTA plasma, and citrate plasma  |   |            |            |
| <b>Standard range</b>  | 0 to 320 pmol/l (7 standards and 2 controls in a human serum matrix. (Standards: 0/10/20/40/80/160/320 pmol/l))   |   |            |            |
| <b>Conversion factor</b>   | 1 pg/ml = 0.044 pmol/l (MW: 22.5 kDa)<br>1 pmol/l = 22.5 pg/ml  |   |            |            |
| <b>Sample volume</b>   | 20 µl / well  |   |            |            |
| <b>Incubation time, temp.</b>  | 2 h / 1 h / 30 min, room temperature  |   |            |            |
| <b>Sensitivity</b>   | LOD: (0 pmol/l + 3 SD): 1.9 pmol/l; LLOQ: 1.3 pmol/l  |   |            |            |
| <b>Specificity</b>   | This assay recognizes endogenous and recombinant human bioactive Sclerostin.  |   |            |            |
| <b>Precision</b>   | Intra-assay (n=3) ≤ 1%, Inter-assay (n=7) ≤ 5%  |   |            |            |
| <b>Spike/Recovery</b>  | Average % recovery spiked with 26 and 110 pmol/l, respectively  | Serum (n=5): 93%, 86%<br>EDTA plasma (n=5): 94%, 93%<br>Citrate plasma (n=1): 104%, 99% |            |            |
| <b>Dilution linearity of recombinant bioactive Sclerostin</b>        | <u>Average % of expected of dilution:</u>   | <u>1+1</u>  | <u>1+3</u> | <u>1+7</u> |
|  | Serum (n=6):  | 98  | 86         | 89         |
|  | EDTA plasma (n=6):  | 102   | 99         | 91         |
|  | Citrate plasma (n=1):   | 119   | 132        | 103        |
| <b>Dilution linearity of endogenous bioactive Sclerostin</b>         | <u>Average % of expected of dilution:</u>   | <u>1+1</u>  | <u>1+3</u> | <u>1+7</u> |
|  | Serum (n=7):  | 100   | 103        | 106        |
|  | EDTA plasma (n=6):  | 105   | 108        | 123        |
|  | Citrate plasma (n=2):   | 91  | 91         | 103        |
| <b>Bioactive Sclerostin values of apparently healthy individuals</b> | <p>Median serum (n=32): 61.5 pmol/l<br/>Median EDTA plasma (n=24): 87 pmol/l<br/>Median citrate plasma (n=24): 61.5 pmol/l</p> <p>Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.</p> |   |            |            |

### TYPICAL STANDARD CURVE



### PRINCIPLE OF THE ASSAY



CAB Capture Antibody: recombinant human monoclonal antibody

DAB Detection Antibody: polyclonal goat antibody

STD Standard: recombinant human bioactive Sclerostin protein (AA24-AA213)  
in human serum

Detailed information on the antibodies utilized in this ELISA can be found on pages 12-13.

## SAMPLE VALUES

### bioactive Sclerostin levels in an apparently healthy cohort

|                | Serum<br>(n=32) | EDTA plasma<br>(n=24) | Citrate plasma<br>(n=24) |
|----------------|-----------------|-----------------------|--------------------------|
| Mean           | 70.8            | 103.9                 | 72.8                     |
| <b>Median</b>  | 61.5            | 87                    | 61.5                     |
| Percentile 95% | 143.4           | 225.8                 | 165.3                    |
| Percentile 5%  | 12.5            | 29.2                  | 19.2                     |
| Minimum        | 8               | 27                    | 18                       |
| Maximum        | 183             | 235                   | 166                      |

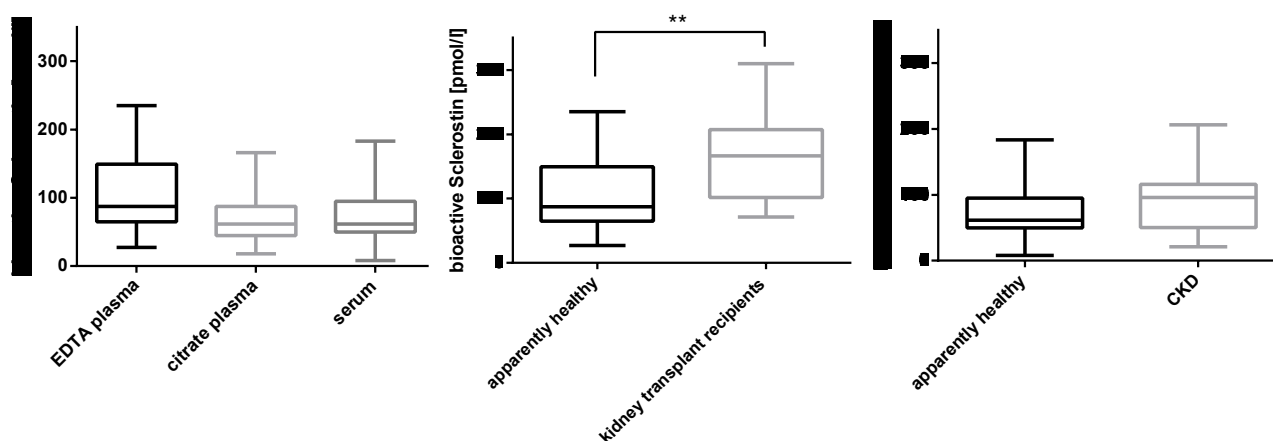
*It is recommended to establish the normal range for each laboratory.*

### Plasma bioactive Sclerostin values in kidney transplant recipients

|                | apparently healthy subjects<br>(n=24) | kidney transplant recipients<br>(n=16) |
|----------------|---------------------------------------|--|
| Mean           | 103.9                                 | 170.3                                  |
| <b>Median</b>  | <b>87</b>                             | <b>166.5</b>                           |
| Percentile 95% | 225.8                                 | 310                                    |
| Percentile 5%  | 29.25                                 | 71                                     |
| Minimum        | 27                                    | 71                                     |
| Maximum        | 235                                   | 310                                    |

### Serum bioactive Sclerostin values in a CKD patient cohort

|                | apparently healthy subjects<br>(n=32) | CKD<br>(n=24) |
|----------------|---------------------------------------|---------------|
| Mean           | 70.8                                  | 94.1          |
| <b>Median</b>  | 61.5                                  | 96            |
| Percentile 95% | 143.4                                 | 200.3         |
| Percentile 5%  | 12.5                                  | 22.7          |
| Minimum        | 8                                     | 21            |
| Maximum        | 183                                   | 206           |



### Why is heparin plasma not suggested as a sample matrix in this ELISA?

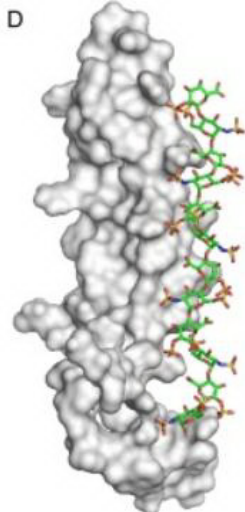


Fig.1: Heparin (green) mainly binds on loop2 and loop3 of the Sclerostin molecule (3).

Heparin disturbs the binding of the detection antibody utilized in this ELISA assay. For this reason, heparin-plasma cannot be measured with this assay.

### MATRIX COMPARISON

#### Comparison of bioactive Sclerostin serum and plasma sample values from apparently healthy individuals

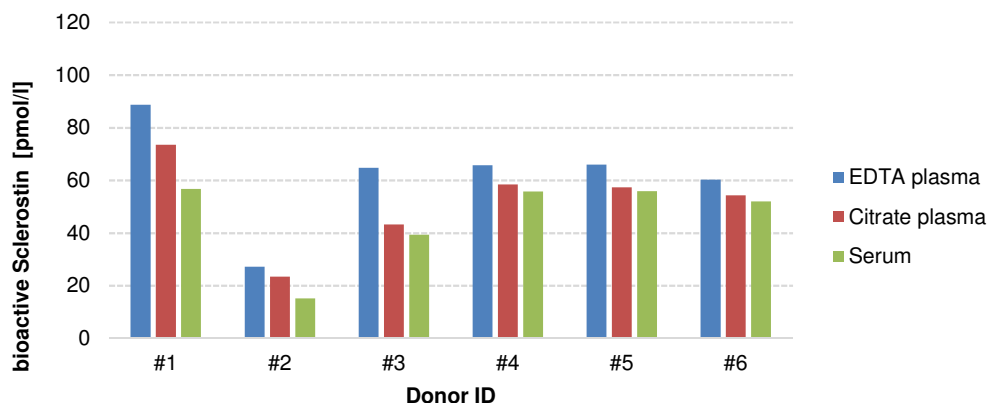
Bioactive human Sclerostin was measured in three matrices from six different individual donors.

| Donor ID | bioactive Sclerostin [pmol/l] |                |       |
|----------|-------------------------------|----------------|-------|
|          | EDTA plasma                   | Citrate plasma | Serum |
| #1       | 89                            | 74             | 57    |
| #2       | 27                            | 23             | 15    |
| #3       | 65                            | 43             | 39    |
| #4       | 66                            | 58             | 56    |
| #5       | 66                            | 57             | 56    |
| #6       | 60                            | 54             | 52    |

Measured values of human bioactive Sclerostin in serum are lower compared to plasma in an apparently healthy cohort (n=6).

It has been shown that Sclerostin values differ between serum and plasma even when these assays are validated in both matrices (5). Measurements of Sclerostin in plasma are generally higher than in serum. The reasons for this difference is yet unclear, however it is assumed that coagulation process under conditions of serum collection might reduce the accessibility of recognizable determinants (6).

Graph showing matrix comparison of bioactive Sclerostin sample concentrations between serum, EDTA plasma, and citrate plasma in an apparently healthy cohort (n=6).



## ASSAY PERFORMANCE CHARACTERISTICS

### SPIKE RECOVERY

Summary of data showing mean recovery of bioactive Sclerostin:

| Matrix               | +26 pmol/l |         | +110 pmol/l |        |
|----------------------|------------|---------|-------------|--------|
|                      | Mean       | Range   | Mean        | Range  |
| Serum (n=5)          | 93%        | 76-111% | 86%         | 82-95% |
| EDTA plasma (n=5)    | 94%        | 85-104% | 93%         | 86-98% |
| Citrate plasma (n=1) | 104%       | -       | 99%         | -      |

Experiments:

Recovery of spiked samples was tested by adding 2 concentrations of human recombinant bioactive Sclerostin (26 pmol/l + 110 pmol/l) to different human sample matrices.

Data showing spike/recovery of human **serum** samples:

| Sample ID       | bioactive Sclerostin [pmol/l] |            |             | S/R [%]    |             |
|-----------------|-------------------------------|------------|-------------|------------|-------------|
|                 | Reference                     | +26 pmol/l | +110 pmol/l | +26 pmol/l | +110 pmol/l |
| #S1             | 57                            | 77         | 147         | 76         | 82          |
| #S2             | 75                            | 104        | 180         | 111        | 95          |
| #S3             | 55                            | 77         | 148         | 84         | 84          |
| #S4             | 44                            | 67         | 140         | 90         | 87          |
| #S5             | 71                            | 97         | 164         | 101        | 84          |
| <b>Mean [%]</b> |                               |            |             | <b>93</b>  | <b>86</b>   |

Data showing spike/recovery of human **EDTA plasma** samples:

| Sample ID       | bioactive Sclerostin [pmol/l] |            |             | S/R [%]    |             |
|-----------------|-------------------------------|------------|-------------|------------|-------------|
|                 | Reference                     | +26 pmol/l | +110 pmol/l | +26 pmol/l | +110 pmol/l |
| #E1             | 190                           | 212        | 295         | 85         | 96          |
| #E2             | 152                           | 177        | 253         | 95         | 92          |
| #E3             | 165                           | 190        | 259         | 97         | 86          |
| #E4             | 81                            | 108        | 189         | 104        | 98          |
| #E5             | 67                            | 91         | 171         | 91         | 94          |
| <b>Mean [%]</b> |                               |            |             | <b>94</b>  | <b>93</b>   |

Data showing spike/recovery of human **citrate plasma** samples:

| Sample ID | bioactive Sclerostin [pmol/l] |            |             | S/R [%]    |             |
|-----------|-------------------------------|------------|-------------|------------|-------------|
|           | Reference                     | +26 pmol/l | +110 pmol/l | +26 pmol/l | +110 pmol/l |
| #C1       | 90                            | 117        | 199         | 104        | 99          |

## LINEARITY

### Summary:

#### Dilution linearity of samples containing endogenous Sclerostin

| Matrix               | Recovery of dilution steps [%] |        |      |        |      |         |
|----------------------|--------------------------------|--------|------|--------|------|---------|
|                      | 1+1                            |        | 1+3  |        | 1+7  |         |
|                      | Mean                           | Range  | Mean | Range  | Mean | Range   |
| Serum (n=7)          | 100                            | 89-108 | 103  | 96-108 | 106  | 90-120  |
| EDTA plasma (n=6)    | 105                            | 99-111 | 108  | 99-125 | 123  | 107-154 |
| Citrate plasma (n=2) | 91                             | 89-94  | 91   | 86-96  | 103  | 102-104 |

#### Dilution linearity of samples containing recombinant bioactive Sclerostin

| Matrix               | Recovery of dilution steps [%] |        |      |        |      |        |
|----------------------|--------------------------------|--------|------|--------|------|--------|
|                      | 1+1                            |        | 1+3  |        | 1+7  |        |
|                      | Mean                           | Range  | Mean | Range  | Mean | Range  |
| Serum (n=6)          | 98                             | 93-103 | 86   | 73-100 | 89   | 75-103 |
| EDTA plasma (n=6)    | 102                            | 97-106 | 99   | 97-103 | 91   | 91-109 |
| Citrate plasma (n=1) | 119                            | -      | 132  | -      | 103  | -      |

› **All samples were diluted in assay buffer provided in the kit.**

#### Experiment:

Dilution linearity was assessed by serially diluting samples containing endogenous bioactive Sclerostin with assay buffer.

Data showing the dilution of endogenous bioactive Sclerostin in **serum** samples:

| Sample ID         | bioactive Sclerostin [pmol/l] |     |     |     | R [%]      |            |            |
|-------------------|-------------------------------|-----|-----|-----|------------|------------|------------|
|                   | ref                           | 1+1 | 1+3 | 1+7 | 1+1        | 1+3        | 1+7        |
| #S1               | 139                           | 67  | 33  | 17  | 97         | 96         | 95         |
| #S2               | 122                           | 60  | 33  | 18  | 98         | 108        | 120        |
| #S3               | 114                           | 61  | 29  | 13  | 108        | 101        | 90         |
| #S4               | 139                           | 75  | 39  | 20  | 108        | 112        | 116        |
| #S5               | 103                           | 50  | 28  | 15  | 98         | 108        | 116        |
| #S6               | 199                           | 88  | 48  | 25  | 89         | 96         | 101        |
| #S7               | 89                            | 46  | 23  | 12  | 104        | 105        | 106        |
| <b>Mean R [%]</b> |                               |     |     |     | <b>100</b> | <b>103</b> | <b>106</b> |

Data showing the dilution of endogenous bioactive Sclerostin in **EDTA plasma** samples:

| Sample ID         | bioactive Sclerostin [pmol/l] |     |     |     | R [%]      |            |            |
|-------------------|-------------------------------|-----|-----|-----|------------|------------|------------|
|                   | ref                           | 1+1 | 1+3 | 1+7 | 1+1        | 1+3        | 1+7        |
| #E1               | 268                           | 147 | 66  | 36  | 110        | 99         | 107        |
| #E2               | 210                           | 109 | 52  | 30  | 104        | 99         | 115        |
| #E3               | 173                           | 87  | 50  | 28  | 100        | 116        | 131        |
| #E4               | 184                           | 98  | 47  | 28  | 106        | 102        | 122        |
| #E5               | 148                           | 82  | 46  | 28  | 111        | 125        | 154        |
| #E6               | 242                           | 120 | 66  | 32  | 99         | 110        | 107        |
| <b>Mean R [%]</b> |                               |     |     |     | <b>105</b> | <b>108</b> | <b>123</b> |

Data showing the dilution of endogenous bioactive Sclerostin in **citrate plasma** samples:

| Sample ID         | bioactive Sclerostin [pmol/l] |     |     |     | R [%]     |           |            |
|-------------------|-------------------------------|-----|-----|-----|-----------|-----------|------------|
|                   | ref                           | 1+1 | 1+3 | 1+7 | 1+1       | 1+3       | 1+7        |
| #C1               | 175                           | 82  | 42  | 23  | 94        | 96        | 104        |
| #C2               | 171                           | 76  | 37  | 22  | 89        | 86        | 102        |
| <b>Mean R [%]</b> |                               |     |     |     | <b>91</b> | <b>91</b> | <b>103</b> |

Experiment:

Dilution linearity was assessed by serially diluting samples containing 110 pmol/l recombinant bioactive Sclerostin with assay buffer.

Data showing the dilution of recombinant bioactive Sclerostin in **serum** samples:

| Sample ID         | bioactive Sclerostin [pmol/l] |     |     |     | R [%]     |           |           |
|-------------------|-------------------------------|-----|-----|-----|-----------|-----------|-----------|
|                   | ref                           | 1+1 | 1+3 | 1+7 | 1+1       | 1+3       | 1+7       |
| #S1               | 147                           | 71  | 37  | 17  | 96        | 100       | 93        |
| #S2               | 180                           | 84  | 37  | 19  | 93        | 82        | 83        |
| #S3               | 259                           | 134 | 62  | 27  | 103       | 96        | 82        |
| #S4               | 148                           | 76  | 31  | 19  | 103       | 85        | 100       |
| #S5               | 140                           | 67  | 27  | 18  | 96        | 78        | 103       |
| #S6               | 164                           | 78  | 30  | 15  | 95        | 73        | 75        |
| <b>Mean R [%]</b> |                               |     |     |     | <b>98</b> | <b>86</b> | <b>89</b> |



Data showing the dilution of recombinant bioactive Sclerostin in **EDTA plasma** samples:

| Sample ID         | bioactive Sclerostin [pmol/l] |     |     |     | R [%]      |           |           |
|-------------------|-------------------------------|-----|-----|-----|------------|-----------|-----------|
|                   | ref                           | 1+1 | 1+3 | 1+7 | 1+1        | 1+3       | 1+7       |
| #E1               | 295                           | 151 | 72  | 35  | 102        | 97        | 96        |
| #E2               | 184                           | 91  | 45  | 21  | 99         | 98        | 93        |
| #E3               | 253                           | 128 | 62  | 29  | 101        | 97        | 91        |
| #E4               | 259                           | 136 | 67  | 32  | 105        | 103       | 97        |
| #E5               | 189                           | 101 | 46  | 22  | 106        | 97        | 95        |
| #E6               | 171                           | 83  | 44  | 23  | 97         | 103       | 109       |
| <b>Mean R [%]</b> |                               |     |     |     | <b>102</b> | <b>99</b> | <b>97</b> |

Data showing the dilution of recombinant bioactive Sclerostin in **citrate plasma** samples:

| Sample ID | bioactive Sclerostin [pmol/l] |     |     |     | R [%] |     |     |
|-----------|-------------------------------|-----|-----|-----|-------|-----|-----|
|           | ref                           | 1+1 | 1+3 | 1+7 | 1+1   | 1+3 | 1+7 |
| #C1       | 174                           | 103 | 58  | 22  | 119   | 132 | 103 |

### Recommendations for sample dilution

High measuring samples outside of the calibration range of the curve should be **diluted with ASYBUF** (assay buffer, supplied in the kit).

### PRECISION

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

Intra-assay (n=3) ≤ 1%, Inter-assay (n=7) ≤ 5%

Intra-assay: 2 samples of known concentrations were tested 3 times with 1 assay lot by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 7 times with 2 assay lots by 2 different operators.

| Intra-assay (n=3) | Sample 1 | Sample 2 | Inter-assay (n=7) | Sample 1 | Sample 2 |
|-------------------|----------|----------|-------------------|----------|----------|
| Mean (pmol/l)     | 19       | 153      | Mean (pmol/l)     | 19       | 157      |
| SD (pmol/l)       | 0.3      | 1.0      | SD (pmol/l)       | 1.0      | 8.3      |
| CV (%)            | 1        | 1        | CV (%)            | 5        | 5        |

### 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 for the bioactive Sclerostin ELISA is **1.9 pmol/l**.

### Lower limit of quantification (LLOQ)

The lower limit of quantification is defined as the lowest concentration where the following two criteria are met: 1) back fit of the calibration standards shall be within 75 – 125% and 2) precision shall be ≤ 25% (acc. to ICH [Ref. 1]).

The LLOQ for the bioactive Sclerostin ELISA is **1.3 pmol/l**.

### SAMPLE STABILITY

#### Sample preparation

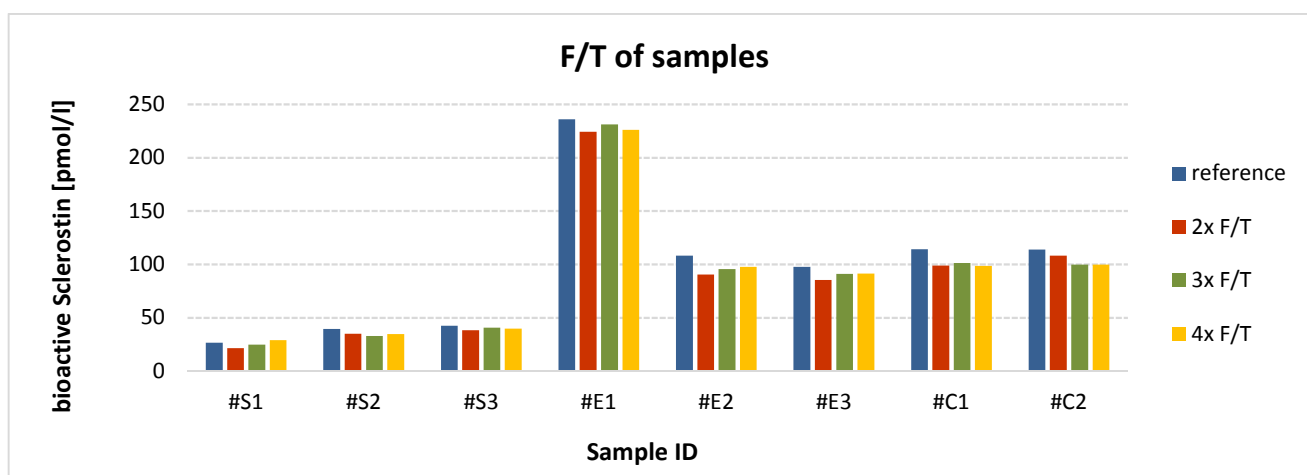
Collect venous blood samples by using standardized blood collection tubes. Perform serum/plasma separation by centrifugation according to supplier's instructions of the blood collection devices as soon as possible. The acquired plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. All samples should undergo only 4 freeze-thaw cycles.

#### Freeze/thaw stability of serum samples containing endogenous bioactive Sclerostin

A set of samples (3 sera, 3 EDTA plasma, 2 citrate plasma) was aliquoted and freeze-thaw stressed. The reference samples are freeze thawed once. Samples can undergo 4 freeze-thaw cycles. The mean recovery of sample concentrations stressed by 4 F/T cycles is 93%.

Sclerostin concentrations of samples after freeze-thaw cycles:

| Sample ID | bioactive Sclerostin [pmol/l] |     |     |                   | R [%]        |
|-----------|-------------------------------|-----|-----|-------------------|--------------|
|           | reference                     | 2x  | 3x  | 4x                | 4 F/T vs ref |
| #S1       | 27                            | 22  | 25  | 29                | 109          |
| #S2       | 40                            | 35  | 33  | 35                | 87           |
| #S3       | 42                            | 38  | 41  | 40                | 94           |
| #E1       | 236                           | 224 | 231 | 226               | 96           |
| #E2       | 108                           | 90  | 95  | 98                | 90           |
| #E3       | 98                            | 85  | 91  | 91                | 93           |
| #C1       | 114                           | 99  | 101 | 99                | 86           |
| #C2       | 114                           | 108 | 100 | 100               | 88           |
|           |                               |     |     | <b>Mean R [%]</b> | <b>93</b>    |



### **SPECIFICITY**

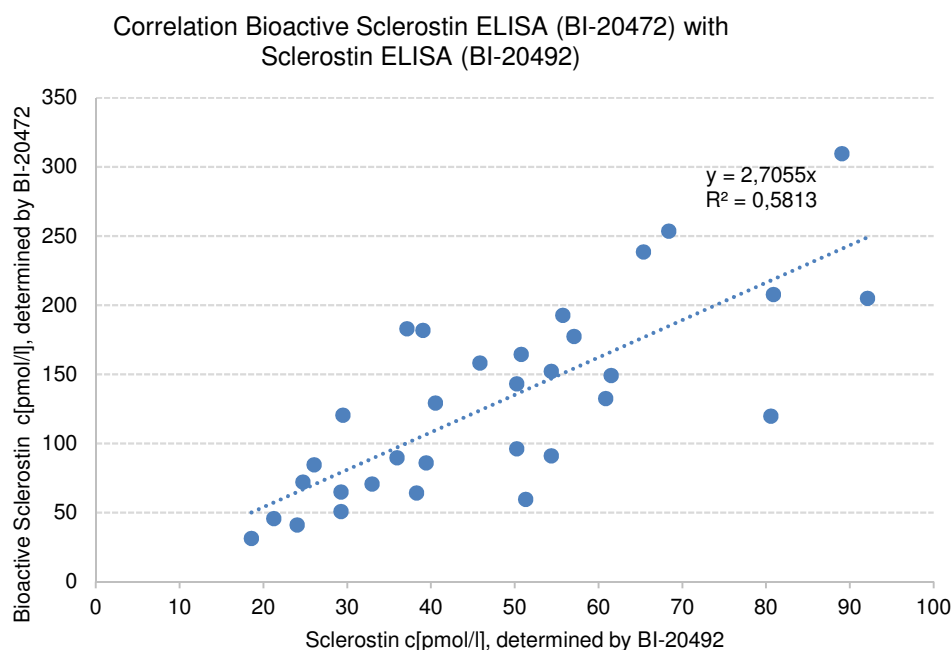
This assay recognizes endogenous (natural) and recombinant human bioactive Sclerostin.

### **CALIBRATION**

This immunoassay is calibrated against recombinant human bioactive Sclerostin (AA24-213).

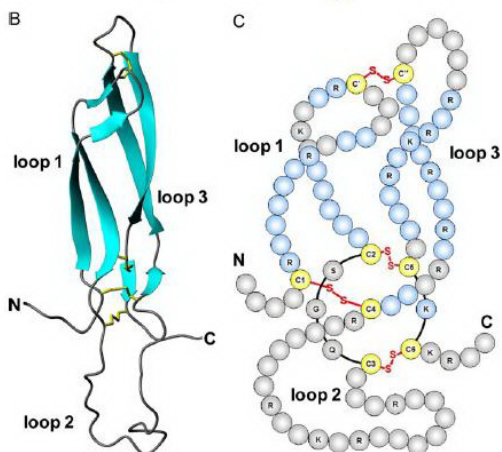
### **COMPARISON of Biomedica' bioactive Sclerostin ELISA (cat.no. BI-20472) with Biomedica's Sclerostin ELISA (cat.no. BI-20492)**

32 samples were compared: EDTA plasma samples (n=16), serum samples (n=16)



**Results:** The correlation between the two assays resulted in  $R^2=0.58$ . Sclerostin sample values measured with the Biomedica "bioactive Sclerostin ELISA" (cat no BI-20472) are higher than in the Biomedica "Sclerostin ELISA" (cat no BI-20492). The results demonstrate that the antibodies utilized in both assays bind to different regions of the Sclerostin molecule. The monoclonal capture antibody of the bioactive Sclerostin ELISA binds to the receptor binding site of Sclerostin; a region that is most probably more robust to cleavage. More information on the characterization of the antibodies see below.

## CHARACTERIZATION OF THE ANTIBODIES utilized in the bioactive Sclerostin ELISA



### Loop 2 – binding region to LRP5/6 = „active“ site (3,4)

Fig.2: Sclerostin (<http://www.uniprot.org/uniprot/Q9BQB4.1>)

The Sclerostin protein consists of two flexible N- and C-terminal arms and a cystine-knot with three loops, whereas the second loop binds to the LRP5/6 complex of the Wnt-signaling pathway and leads to the inhibition of bone formation (3, 4).

Sclerostin is classically considered to be a monomeric protein, but data from *Hernandez and colleagues* (7) postulate that circulating sclerostin has a dimeric configuration. Furthermore, it is not yet well understood if circulating Sclerostin fragments exist, but the comparison of different ELISAs suggest that those fragments exist as well (8, 9).

As the epitope of the monoclonal capture antibody utilized in the *bioactive Sclerostin ELISA* is located in loop 2 (see Fig.2), the binding region to the LRP 5/6 complex, all Sclerostin molecules (including potential fragments) containing this receptor binding region can be detected.

The characterization of both antibodies utilized in the *bioactive Sclerostin ELISA* comprises epitope mapping with overlapping peptides spotted to a microarray, characterization of binding kinetics with biolayer interferometry measurements and determination of antibody purity with size exclusion chromatography.

### Sclerostin Protein Structure - EPITOPES OF COATING AND DETECTION ANTIBODY

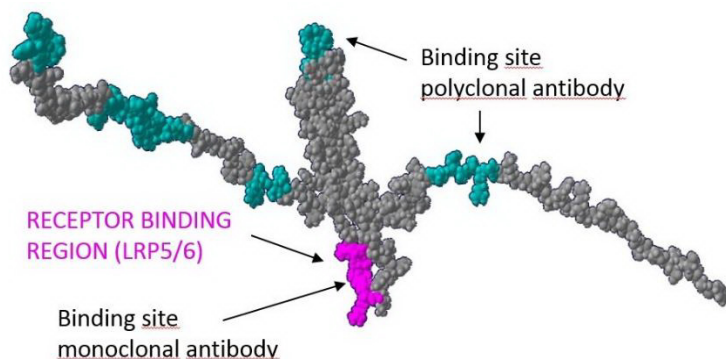


Fig.3: Sclerostin protein structure showing the binding regions of the monoclonal capture antibody (pink) and the polyclonal detection antibody (turquoise.)

## AFFINITY OF COATING AND DETECTION ANTIBODY

Both ELISA antibodies utilized in the "bioactive Sclerostin ELISA" bind to Sclerostin with high affinity.

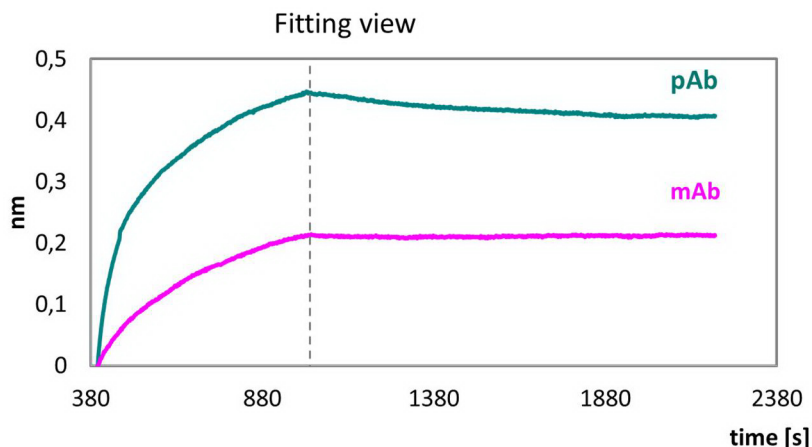


Fig.4: Biolyer interferometry measurements (Octet) of monoclonal coating antibody (mAb, pink) and polyclonal detection antibody (pAb, turquoise) binding to a sensor coated with sclerostin protein.

## HPLC ANALYSIS OF COATING AND DETECTION ANTIBODY

### HPLC analysis reveals >95% purity of antibody monomers

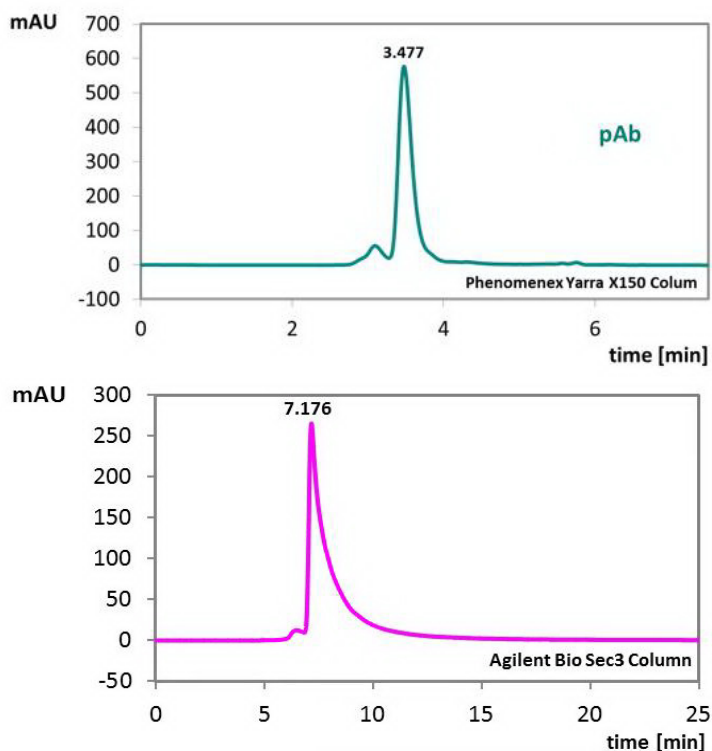


Fig.5: HPLC analysis of both antibodies. Size exclusion chromatography (SEC) of monoclonal antibody (mAb, pink) and polyclonal antibody (pAb, turquoise). The monoclonal antibody was analyzed using an Agilent Bio Sec column, whereas for the polyclonal antibody a Phenomenex Yarra X150 column was used.

## **VALIDATION GUIDELINES AND LITERATURE**

This document is based on the principles of bioanalytical validation defined by ICH Ref. (1), (2) and according to SOP A801.

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”
2. Food and Drug Administration  
Guidance for Industry, Bioanalytical Method Validation, Draft Guidance, September 2013  
Guidance for industry (Draft status), Bioanalytical Validation, FDA Sept2013, Revision 1,  
chapter IV.
3. Characterization of the structural features and interactions of sclerostin: molecular insight  
into a key regulator of Wnt-mediated bone formation. *Veverka V et al., J Biol Chem, 2009;  
284:10890-10900.*
4. Characterization of the Interaction of Sclerostin with the Low Density Lipoprotein Receptor-  
related Protein (LRP) Family of Wnt Co-receptors. *Holdsworth G et al., J Biol Chem, 2012;  
284(16), 287(32): 26464-26477.*
5. Determination of serum and plasma sclerostin concentrations by enzyme-linked  
immunoassays. *McNulty M et al., J Clin Endocrinol Metab, 2012; 96 (7), E1159-E1162*
6. Sclerostin measurement in human disease: Validity and current limitations. *Costa A et al.,  
Bone, 2017; 96:24-28.*
7. New insights into the location and form of sclerostin. *Hernandez P et al., Biochem Biophys  
Res Commun, 2014; 446 (4):1108-1113.*
8. Association of circulating sclerostin with bone mineral mass, microstructure, and turnover  
biochemical markers in healthy elderly men and women. *Durosier C et al., J Clin Endocrinol  
Metab, 2013; 98 (9):3873-3883.*
9. Circulating sclerostin levels are decreased in patients with endogenous hypercortisolism and  
increase after treatment. *van Lierop AH et al., J Clin Endocrinol Metab, 2012; 97:E1953-  
E1957.*

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Instructions for Use (package insert)

Material Safety Data Sheet

**MEASUREMENT of BIOACTIVE SCLEROSTIN in CELL CULTURE SUPERNATANTS and URINE SAMPLES**

The following experiments have been performed to test the use of the bioactive Sclerostin assay (cat. no. BI-20472) in human urine and cell culture supernatants.

Note: the experiments performed for these samples did not undergo a full validation and are therefore merely a performance check.

**1. MEASUREMENT of bioactive Sclerostin in CELL CULTURE SUPERNATANTS**

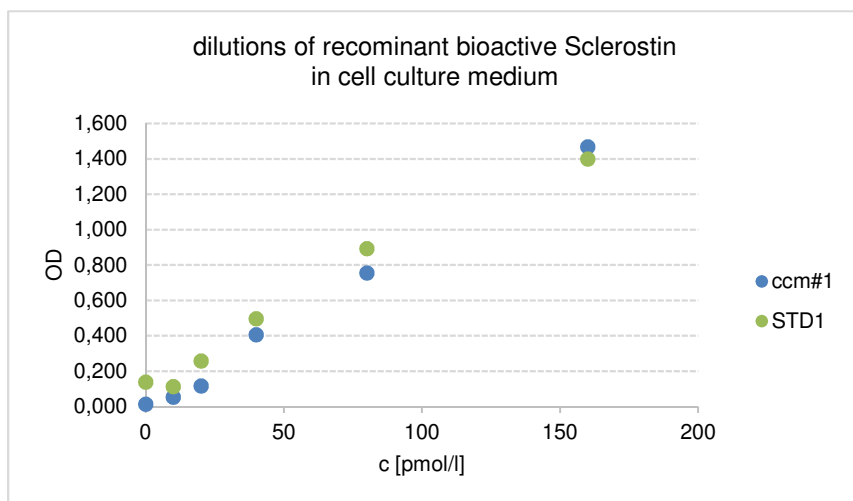
Cell culture medium (ccm: RPMI1640 containing 10% fetal calf serum) was tested undiluted and spiked with a final concentration of 160 pmol/l human Sclerostin protein. The spiked solution was diluted 1+1, 1+3, 1+7, and 1+15 with the cell culture medium.

As a comparison, the spike recovery and dilution linearity of the standard matrix (=STD1) and the dilutions with assay buffer is shown.

OD values of spiked and diluted cell culture medium sample and standard matrix (STD1)

| Dil medium | Sample ID | OD        |              |       |       |       |       |
|------------|-----------|-----------|--------------|-------|-------|-------|-------|
|            |           | Reference | + 160 pmol/l | 1+1   | 1+3   | 1+7   | 1+15  |
| ccm#1      | ccm#1     | 0.014     | 1.468        | 0.756 | 0.406 | 0.116 | 0.054 |
| ASYBUF     | STD1      | 0.138     | 1.400        | 0.892 | 0.496 | 0.258 | 0.113 |

Graph showing dilution of cell culture medium (ccm) and a comparison to the standard Matrix (STD1), both spiked with spiked with the same amount of recombinant Sclerostin (160 pmol/l).



**Suggested protocol for the measurement of human bioactive Sclerostin in cell culture supernatants**

Preparation of a cell culture medium (ccm) based standard curve:

Reconstitute STD7 in 250 µl deionized water. Leave at room temperature (18-26°C) for 15 min and mix well prior to making dilutions.

*Use polypropylene tubes.*

For the preparation of the cell culture based standards *always* use the identical cell culture medium in which the samples are based on.

- Mark tubes e.g. CC STD6, CC STD 5 ... CC STD1.
- Prepare a two-fold serial dilution to obtain STD6 to STD2.

e.g.:

Dispense 100 µl cell culture medium into vials labelled with CC STD6 to CC STD1.

Pipette 100 µl of STD 7 into tube marked as CC STD6. Mix thoroughly.

Transfer 100 µl of CC STD6 into vial marked as CC STD5. Mix thoroughly. Continue in the same fashion to obtain CC STD4 to CC STD2.

- ccm serves as the zero standard (=CC STD1, 0 pmol/l).

*Attention: Concentrations defined for CTRL A and B are only valid for measuring bioactive Sclerostin in human serum or plasma. The controls cannot be used for cell culture measurements.*

## **2. MEASUREMENT of bioactive Sclerostin in human URINE**

The Biomedica ELISA is fully validated for the measurement of human bioactive Sclerostin in serum, EDTA-, and citrate plasma.

The ELISA has not been fully validated for the measurement of bioactive Sclerostin in urine samples.

A small number of experiments with urine samples have been performed

### **Summary:**

Urine samples (n=4) were assayed with the bioactive Sclerostin ELISA (BI-20472) following the standard protocol using undiluted urine.

Endogenous bioactive Sclerostin was not detectable in these samples.

Urine samples can be spiked:

The average recovery of 4 human urine samples from hospital donors was 103%.

If required, dilute urine samples 1+1 with ASYBUF (Assay buffer, supplied in the kit).



**Suggested protocol for the measurement of human bioactive Sclerostin in urine samples**

Follow standard protocol as indicated in the package insert:

Pipette **20 µl** of **undiluted urine sample** directly into the well of the microtiter plate.

If required, dilute samples 1+1 with assay buffer (provided in the kit).