TECOmedical Group

TECO®
Sclerostin HS

Human Sclerostin HS ELISA

Instructions for use
English

For Research Use Only
Not for Use in Diagnostic Procedures.

Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

TE1023-HS

Caution: caustic

Intended use

Tests

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TECO® Sclerostin HS ELISA Kit

CONT Reagents and Materials Supplied:

SYMBOL	DESCRIPTION	FORMAT
1	**96-well plate coated with streptavidin** 12 break apart strips of 8 wells (12 x 8 in total), in a frame with plate cover. Ready to use	**1 plate**
A	**Standard A** 0 ng/ml – 0 pmol/l – Ready to use	**1 x 0.5 ml**
B	**Standard B** 0.025 ng/ml – 1.1 pmol/l – Ready to use	**1 x 0.5 ml**
C	**Standard C** 0.074 ng/ml – 3.3 pmol/l – Ready to use	**1 x 0.5 ml**
D	**Standard D** 0.22 ng/ml – 9.7 pmol/l – Ready to use	**1 x 0.5 ml**
E	**Standard E** 0,67 ng/ml – 29.5 pmol/l – Ready to use	**1 x 0.5 ml**
F	**Standard F** 2 ng/ml – 88 pmol/l – Ready to use	**1 x 0.5 ml**
G	**Standard G** 4 ng/ml – 176 pmol/l – Ready to use	**1 x 0.5 ml**
L	**Control 1** Ready to use, Range as indicated on data sheet	**1 x 0.5 ml**
H	**Control 2** Ready for use, Range as indicated on data sheet	**1 x 0.5 ml**
2	**Wash Buffer** 50 times concentrate	**1 x 30 ml**
3	**Sample Diluent** Ready to use	**1 x 5 ml**
4	**Matrix Solution** Ready to use	**1 x 7 ml**
5	**Antibody Solution** Ready to use	**1 x 7 ml**
7	**TMB Substrate** Ready to use	**1 x 12 ml**
8	**Stop Solution – 1 M HCl** 1 M hydrochloric acid, ready to use	**1 x 12 ml**
	Kit instructions	**1 x**

Storage

Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

Instructions for Use

The TECO® Sclerostin HS kit is a sensitive sandwich enzyme linked immunosorbent assay for the quantitative determination of sclerostin in human serum, plasma and cell culture.

Summary and Explanation

The Human Sclerostin High Sensitivity (HS) Enzyme Immunoassay is a 96-well, direct-capture immunoassay for the measurement of Sclerostin in human serum, plasma and cell culture (osteocytes and chondrocytes). Sclerostin is the protein product of the SOST gene, which is located at 17q12-21 and highly conserved across vertebrate species. The highest expression of sclerostin throughout the adult skeleton has been observed in hypertrophic chondrocytes and osteocytes. Sclerostin blocks canonical Wnt signaling by binding to the Wnt coreceptors LRP5/6, inhibiting bone formation by regulating osteoblast function and promoting osteoblast apoptosis.[1-3] Sclerostin also antagonizes bone morphogenetic protein (BMP) action (e.g. osteoblast differentiation), but does not inhibit direct BMP-induced responses.[4-7] Sclerostin expression is down-regulated by Parathyroid hormone (PTH), as well as, by the mechanical stimulation of bone.[8-12] Reduced expression of sclerostin can result in van Buchem disease, while a complete absence results in Sclerosteosis. Persons affected by Sclerosteosis show progressive hyperostosis and sclerosis of the skull, mandible and all long bones. Bone mineral density (BMD), bone volume, bone formation rate, and bone strength are significantly increased, while overall skeletal morphology appears to be normal.[13-14] A predominance of sclerostin causes reduced bone quality (Osteoporosis pseudoglioma (OPPG) syndrome). Down-regulation of sclerostin might be used as a treatment for diseases such as osteoporosis, promote osseointegration of implants, prevent periprosthetic bone loss, or treat non-union in fractures.[15-20] Local enhancement of sclerostin expression might be used to prevent cancer metastasis and minimize further expansion of ectopic bone formation.[21]

References

1. Ott S.M. 2005. “Sclerostin and Wnt signaling-the pathway to bone strength.” J Clin Endocrinol Metab. 90(12):6392-6395.
2. Semenov M, Tamai K, He X. 2005. “SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor.” J Biol Chem. 280(29):26770-26775.
3. Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris S.E., Wu D. 2005. “Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling.” J Biol Chem. 280(20):19883-19887.
4. ten Dijke P, Kraus C, de Gorter D.J., Lowik C.W., van Bezooijen R.L. 2008. “Osteocyte-derived sclerostin inhibits bone formation: its role in bone morphogenic protein and Wnt signaling.” J Bone Joint Surg Am. 90(Suppl. 1):31-35.
5. Winkler D.G., Sutherland M.K., Geoghegan J.C., Yu C., Hayes T., Skonier J.E., Shpekor D., Jonas M., Kovacevich B.R., Staehling-Hampton K., Appleby M., Brunkow M.E., Latham J.A. 2003. “Osteocyte control of bone formation via sclerostin, a novel BMP antagonist.” The Embo J. 22(23):6267-6276.
6. Krause C., Korchynskyi O., de Rooij K., Weidauer S.E., de Gorter D.J., van Bezooijen R.L., Hatsell S., Economides A.N., Mueller T.D., Lowik C.W., ten Dijke P. 2010. “Distinct modes of inhibition by sclerostin on bone morphogenic protein and Wnt signaling pathways.” J Biol Chem. 285(53):41614-41626.
7. Sutherland M.K., Geoghegan J.C., Yu C., Winkler D.G., Latham J.A. 2004. “Unique regulation of SOST, the sclerosteosis gene, by BMPs and steroid hormones in human osteoblasts.” Bone 35(2):448-454.
8. Keller H., Kneissel M. 2005. “SOST is a target gene for PTH in bone.” Bone 37(2):148-158.
9. Bellido T., Ali A.A., Gubrij I., Plotkin L.I., Fu Q., O’Brien C.A., Manolagas S.C., Jilka R.L. 2005. “Chronic elevation of paraghyroid hormone in mice reduces expression of sclerostin by osteocytes: novel mechanism for hormonal control of osteoblastogenesis.” Endocrinology 146(11):4577-4583.
10. Silvestrini G., Ballanti P., Leopizzi M., Sebastiani M., Berni S., Di Vito M., Bonucci E. 2007. “Effects of intermittent parathyroid hormone (PTH) administration on SOST mRNA and protein in rat bone.” J Molec Hist. 38(4)l261-269.
11. Robling A.G., Bellido T., Turner C.H., 2006. “Mechanical stimulation in vivo reduces osteocyte expression of sclerostin.” J Musculoskelet Neuronal Interact. 6(4):354.
12. Robling A.G., Niziolek P.J., Baldridge L.A., Condon K.W., Allen M.R., Alam I., Mantila S.M., Gluhak-Heinrich J., Bellido T.M., Harris S.E., Turner C.H. 2008. “Mechanical stimulation of bone in vivo reduces osteocyte expression of SOST/Sclerostin.” J Biol Chem. 283(9):5866-5875.
13. Hamersma H., Gardner J., Beighton P. 2003. “The natural history of sclerostosis.” Clin Genet. 63(3)L192-197.
14. van Bezooijen R.L., Bronckers A.L., Gortzak R.A., Hogendoorn P.C.W., van der Wee-Pals L., Balemans W., Oostenbroek H.J., Van Hul W., Hamersma H., Dikkers F.G., Hamdy N.A.T., Papapoulos S.E., Lowik C.W.G.M. 2009. “Sclerostin in mineralized matrices and van Buchem disease.” J Dent Res. 88(6):569-574.
15. Li X., Ominsky M.S., Warmington K.S., Morony S., Gong J., Cao J., Gao Y., Shalhoub V., Tipton B., Haldankar R., Chen Q., Winters A., Boone T., Geng Z., Niu Q-T., Ke H.Z., Kostenuik P.J., Simonet W.S., Lacey D.L., Paszty C. 2009. “Sclerostin antibody treatment increases bone formation, bone mass and bone strength in a rat model of postmenopausal osteoporosis.” J Bone Miner Res. 24(4):578-588.
16. Tian X., Setterberg R.B., Li X., Paszty C., Ke H.Z., Jee W.S.S. 2010. “Treatment with a sclerostin antibody increases cancellous bone formation and bone mass regardless of marrow composition in adult female rats.” Bone 47(3):529-533.
17. Agholme F., Li X., Isaksson H., Ke H.Z., Aspenberg P. 2010. “Sclerostin antibody treatment enhances metaphyseal bone healing in rats.” J Bone Miner Res. 25(11):2412-2418.
18. Padhi D., Jing G., Stouch B., Fang L., Posvar E. 2011. “Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody.” J Bone Miner Res. 26(1):19-26.
19. Tian X., Jee W.S.S., Li X., Pascty C., Ke H.Z. 2011. “Sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model.” Bone 48(2):197-201.
20. Ominsky M.S., Li C., Li X., Tan H.L., Lee E., Barrero M., Asuncion F.J., Dwyer D., Han C-Y., Vlasseros F., Samadfam R., Jolette J., Smith S.Y., Stolina M., Lacey D.L., Simonet W.S., Paszty C., Li G., Ke H.Z. 2011. “Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones.” J Bone Miner Res. 26(5):1012-1021.
21. Ettenberg S.A., Charlat O., Daley M.P., Liu S., Vincent K.J., Stuart D.D., Schuller A.G., Yuan J., Ospina B., Green J., Yu Q., Walsh R., Li S., Schmitz R., Heine H., Bilic S., Ostrom L., Mosher R., Hartlepp K.F., Zhu Z., Fawell S., Yao Y-M., Stover D., Finan P.M., Porter J.A., Sellers W.R., Klagge I.M., Cong F. 2010. “Inhibition of tumorigenesis driven by different Wnt proteins requires blockade of ligand-binding regions by LRP6 antibodies.” Proc Natl Acad Sci. 107(35):15473-15478.

Assay Principle

The TECO® Human Sclerostin HS Enzyme Immunoassay for the quantitation of Sclerostin in human plasma and serum is a two-step procedure utilizing (1) a microwell plate coated with streptavidin and a biotinylated goat polyclonal antibody that binds specifically to human Sclerostin, (2) a HRP-conjugated monoclonal anti-human Sclerostin antibody, and (3) a chromogenic substrate.
Prior to Step 1, The microwell plate is pre-washed for 2 minutes, the wash buffer aspirated and the remaining liquid removed by tapping on absorbent paper.
In Step 1, Standards, Controls, and test specimens are added to microassay wells pre-coated with streptavidin. Biotin-conjugated primary polyclonal anti-human Sclerostin antibody and horseradish peroxidase (HRP)-conjugated secondary monoclonal anti-human Sclerostin antibody is added to each test well. Sclerostin present in the Standards, Controls or specimens are captured in the microassay wells through binding of the biotinylated primary antibody to the streptavidin immobilized on the plate and simultaneously detected by the HRP-conjugated secondary antibody. After a 4 hour incubation, a wash cycle removes unbound material.
In Step 2, a chromogenic enzyme substrate is added to each microassay well. The bound HRP-conjugate reacts with the substrate, forming a blue color. After incubation the enzyme reaction is stopped chemically (4), the color changes to yellow, and the color intensity is measured spectrophotometrically at 450 nm with a 590-650 nm reference filter. The color intensity of the reaction mixture is proportional to the concentration of Sclerostin present in the test specimens, Standards, and Controls.
The standard range of the TECO® Sclerostin ELISA Kit is between 0 and 2 ng/ml. In order to avoid additional sample dilution this kit provides an optional standard range extension up to 4 ng/ml by using an additional measured at 405 nm with a 590-650 nm reference filter.

Materials Required and not Supplied

- Pipettes 10 µl – 1000 µl
- Multichannel pipettes for 50 µl –100 µl
- Graduated cylinders for reconstituting or diluting reagents
- Manual Aspiration System or Automatic washer for ELISA plates
- Distilled or deionized water
- Vortex mixer
- ELISA plate reader suitable for 96 well formats and capable of measuring at 405 nm and 450 nm (Reference: 590-650 nm)
- ELISA plate shaker (500 rpm) (orbital shaker)
- Software package for data generation and analysis

Warnings and Precautions

This kit is intended for in vitro research use by professional persons only.
Follow the instructions carefully.
Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to ”Materials Safety Data Sheet” for more detailed safety information.

The TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1. For Research Use Only. Not for use in diagnostic procedures.
2. Treat all specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any samples.
3. Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy but also these materials should be handled as potentially infectious.
4. Disposal of containers and unused contents should be performed in accordance with federal and local regulatory requirements.
5. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
6. Store assay reagents as indicated.
7. Do not use coated strips if pouch is punctured.
8. Test each sample in duplicate.
9. Use of multichannel pipettes is recommended to ensure the timely delivery of liquids, however, do NOT use a multichannel pipette for plate washing steps.
10. a) 1 M hydrochloric acid is caustic and can cause severe burns.
    b) Handle TMB (3,3',5,5'-tetramethylbenzidine) with care, and minimize exposure to light
    Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.

11. As preservative 5-Bromo-5-nitro-1,3-dioxane (0,06%) is used for the antibody and Sample Diluent. Do not ingest. Avoid contact with skin, eyes, or clothing. If contact is made, wash with water. If ingested, call a physician.

Preparation of Reagents

1 **Microwell Plate Coated with Streptavidin**
12 break apart strips of 8 wells (96 in total) in a frame with cover plate and sealed in a foil bag. Fit strip wells firmly into the frame. After opening, immediately return any unused wells to the original foil package and seal. Store at 2–8 °C until expiration date.

A **Standards**
7 vials containing 0.5 ml recombinant Sclerostin:
G (0, 0.025, 0.074, 0.22, 0.67, 2 and 4 ng/ml) - (0, 1.1, 3.3, 9.7, 29.5, 88, 176 pmol/ml)
Ready to use.
Store at 2–8 °C until expiration date.

L **Control 1**
1 vial of 0.5 ml low control. Ready to use.
Store at 2–8 °C until expiration date.

H **Control 2**
1 vial of 0.5 ml high control. Ready to use.
Store at 2–8 °C until expiration date.

2 **Wash Buffer**
1 vial of 30 ml buffer, 50 x concentrated. Precipitation may occur in the buffer; resolve before use by warming and mixing. Bring the vial content to 1500 ml with deionized or distilled water. The diluted washing solution is stable for 4 weeks at 2–8 °C. Store undiluted buffer at 2–8 °C until expiration date.

3 **Sample Diluent**
1 vial of 5 ml. Ready for use.
Store at 2–8 °C until expiration date.

4 **Matrix Solution**
1 vial of 7 ml. Ready to use.
Store at 2–8 °C until expiration date.

5 **Antibody Solution**
1 vial of 7 ml. Ready to use.
Store at 2–8 °C until expiration date.

7 **TMB Substrate**
1 vial of 12 ml of H_2O_2 and stabilized 3,3',5,5'-tetramethylbenzidine. Ready to use.
Store at 2–8 °C until expiration date.

8 **Stop Solution – 1 M HCl**
1 vial of 12 ml of 1 M hydrochloric acid. Ready to use. Store at 2–8 °C until expiration date.

Cover for microwell plate

Specimen Handling and Preparation

Specimen Collection and Storage

Plasma (Heparin and EDTA) and serum have been used as samples in the TECO® Human Sclerostin HS Assay. Collect specimens using standard venipuncture techniques. Specimens should be collected in such a way to avoid hemolysis. For serum specimens, allow the blood to clot, and separate the serum by centrifugation. Both Heparin and EDTA plasma can be used. See Observed Values section for more information.

Stability of Samples

Samples can be stored for 3 days at room temperature, 5 days at 2-8 °C, at ≤ -20 °C for 24 months and at ≤ -80 °C for > 24 months. Up to three thaw cycles may be performed without affecting the samples. If samples need additional freezing for further analysis, we suggest freezing multiple aliquots of the specimen to prevent exceeding the recommended number of freeze/thaw cycles.

CAUTION: Treat all specimens as potentially infectious. Use Universal Precautions. Do not use contaminated or improperly stored specimens.

Normal Specimens must not be diluted. Specimens with high levels of sclerostin (above the standard curve) may require dilution with Sample Diluent and retesting.

Handle and dispose of all specimens using universal Precautions

Assay Procedure

All determinations (Standards, Controls and samples) should be assayed in duplicate. When performing the assay, Standards, Controls and samples should be pipetted as fast as possible
(<15 minutes).
To avoid distortions due to differences in incubation times, Substrate Solution and Stop Solution should be added to the plate in the same order and with the same time interval.

Allow all reagents to stand at room temperature (20–25 °C) for at least 30 minutes. During all incubation steps, plates should be sealed with the adhesive foil or a plastic cover. For light protection, incubate in a dark chamber or cover plate with aluminum foil.

1. Prepare the microassay strips as follows:
   a. Using a wash bottle or automated plate washing device, add approximately 300 µL Wash Buffer to each well.
   b. Incubate the wells for two minutes at 20-25°C.
   c. Aspirate the contents from each well.
   d. Invert the plate and tap firmly on absorbent paper to
2. remove any remaining liquid. Allocate the wells of the Microwell plate **1** for standards, controls and samples.
3. Pipette 25 µl of each Standard (**A** till **G**), Controls (**L** and **H**) and samples into the corresponding wells.
4. Add 50 µl Matrix Solution **4** in each well (multichannel pipette).
5. Add 50 µl of Antibody Solution **5** (multichannel pipette).
6. Cover the wells with a plastic cover and incubate the plate for 4h ± 5 min at room temperature (20–25 °C) on a shaker (500 rpm).
7. After incubation, aspirate the wells by using a plate washer or manually decant by inverting the plate. Wash the wells 4 times with 350 µl diluted Wash Buffer per well. After the last wash cycle tap the inverted wells on a dry absorbent surface to remove excess wash solution. The use of an automatic plate washer is recommended.
8. Following the last washing step, pipette 100 µl of the TMB Solution **7** in each well (multichannel pipette).
9. Incubate the plate for 20 - 30 min, in the dark, at room temperature (20–25 °C) on a shaker (500 rpm).
10. Stop the reaction by adding 100 µl of Stop Solution **8** (multichannel pipette).
11. Measure the color reaction within 10 minutes at 450 nm and 405nm (reference filter between 590–650 nm)
    Std **A** - **F** at 450 nm for values up to 2 ng/ml.
    Only for higher values from 2ng/ml up to 4 ng/ml use Std. **A** - **G** at 405 nm

Result Analysis

A Standard curve can be established by plotting standard concentration on the x-axis (linear scale) against the absorbance of the Standards on the y-axis (linear scale). A 4-parameter curve fit (y= (A-D)/(1+(x/C)B)+D) should be used for automatic data reduction. Alternatively, a quadratic (polynomial) fit is possible. The Sclerostin concentrations in undiluted samples can then be read off the Standard curve. Sclerostin concentration of pre-diluted samples will be obtained by multiplying the value read off the standard curve by the dilution factor used for the given sample.

For the validity of the assay, the 650 nm corrected absorbance (OD) values of Standard A (0 ng/mL) should be below 0.050 and the absorbance value of Standard F should exceed 1.500.

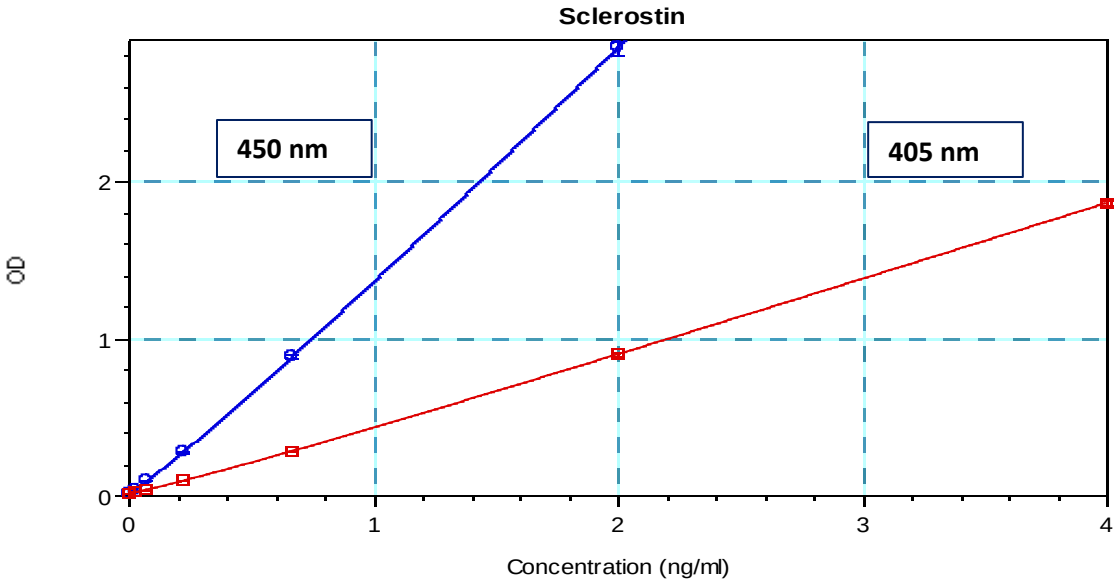
For each assay, the results of the Controls must be within the target range indicated for every lot.
The QC protocol with target ranges is provided with the kit. If control values are not within the limits of the provided range, the assay results should be considered questionable and the samples should be tested again.

Samples with higher absorbance values than Standard G should be tested again with a higher dilution.

Typical Standard Curve and Controls

(Example only. Not for use in calculation of actual results)

Standards	Conc. ng/mL	OD 450 nm	OD 405 nm
A	0	0.010	0.011
B	0.025	0.036	-
C	0.074	0.096	0.039
D	0.22	0.273	0.093
E	0.67	0.880	0.281
F	2	2.284	0.898
G	4	-	1.859



4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
Std1 (STD#1: Conc. vs MeanValue)	0.012	1.09	112	227	1
Std2 (STD#2: Concentration vs MeanValue)	0.0127	1.1	57.8	36.8	1

Weighting: Fixed

Observed Values

Serum from normal donors were tested in the Human Sclerostin HS Enzyme Immunoassay kit. The results are presented below.

Group	n	Mean (ng/mL)	SD
Premenopausal women	19	0.41	0.13
Postmenopausal women	18	0.63	0.19
Men	18	0.86	0.35

NOTE: The mean and Standard Deviation (SD) behaviour of Sclerostin concentrations determined for serum samples may vary between laboratories. Therefore, it is recommended that each laboratory determine the mean Sclerostin concentration and standard deviation values for samples.

Sample	n	Mean (ng/mL)	SD
Serum	9	1.15	0.78
Heparin plasma	9	1.34	0.88
EDTA plasma	9	1.37	0.90

NOTE: 20% higher Sclerostin values have been observed in Heparin and EDTA plasma, compared with serum.

Test Performance

Calibration

The Standards in the kit are prepared from recombinant Sclerostin.
Conversion of ng/ml to pmol/l
1 ng/ml = 44 pmol/l (MW: 22.5 kDa)

LOD: The limit of detection (LOD) for the Sclerostin HS ELISA is 0.006 ng/mL, determined by the upper 3SD limit in a zero standard study.
LLOQ: The lower limit of quantitation (LLOQ) for the Sclerostin HS ELISA is 0.025 ng/mL, the lowest concentration on the standard curve that met CLSI criteria for accuracy and precision.
ULOQ: The upper limit of quantitation (ULOQ) for the Sclerostin HS ELISA is 4.0 ng/mL, the highest concentration that met CLSI criteria for accuracy and precision.

Precision
(Inter assay)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Sample 1	0,40	0,01	2,0
Sample 2	0,29	0,01	4,8
Sample 3	0,88	0,01	1,6
Sample 4	0,31	0,01	2,9
		Mean	**2,8**

(Intra assay)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Sample 1	2,31	0,10	4,2
Sample 2	1,74	0,08	4,3
Sample 3	1,74	0,03	1,7
Sample 4	1,22	0,03	2,2
Sample 5	0,20	0,02	8,2
Sample 6	0,24	0,01	6,1
		Mean	**4,8**

Linearity (Dilution recovery)

Sample	Dilution	Measured (ng/ml)	Expected (ng/ml)	Recovery %
Sample 1	1 2 4	1,137 0,549 0,262	 0,569 0,284	 **97** **92**
Sample 2	1 2 4	0,561 0,260 0,134	 0,281 0,140	 **93** **96**
Sample 3	1 2 4	0,710 0,387 0,145	 0,355 0,178	 **109** **82**
Sample 4	1 2 4 8	1,790 0,798 0,357 0,176	 0,895 0,448 0,224	 **89** **115** **79**
Sample 5	1 2 4	0,271 0,137 0,066	 0,136 0,068	 **101** **97**
			Mean	**95,4**
			SD	**11,2**

Spike recovery

Sample	Concentration (ng/ml)	Added (ng/ml)	Expected (ng/ml)	Measured (ng/ml)	Spike Recovery %
DB	0	0,714 0,178			
Sample 1	1,137	0,714 0,178	1,851 1,315	1,621 1,213	88 92
Sample 2	0,561	0,714 0,178	1,275 0,739	1,261 0,700	99 95
Sample 3	0,710	0,714 0,178	1,424 0,888	1,419 0,870	100 98
Sample 4	1,790	0,714 0,178	2,504 1,968	2,323 1,793	93 91
Sample 5	0,271	0,714 0,178	0,985 0,449	1,010 0,437	103 97
				Mean	**95,5**
				SD	**4,8**

Cross reactions

Serum from different species were tested in Human Sclerostin HS Assay. Results are provided below.

Sample	Sclerostin (ng/mL)*
Bovine	0.00
Goat	0.00
Canine	0.13
Chicken	0.06
Guinea Pig	0.00
Rabbit	0.00
Rat	0.00
Mouse	0.00
Baboon	0.35
Female Cynomolgus Monkey	0.10
Sheep	0.00
Pig	0.00
Rhesus Monkey	0.36
African Green Monkey	0.00
Male Cynomolgus Monkey	0.59

*Concentration >0.10 ng/mL = Cross-reactivity

Interference

The following substances were tested in the Sclerostin HS EIA and found to not interference with the assay using serum samples:

Substance	Concentration
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Glucose	1200 mg/dL
Cholesterol	500 mg/dL
Albumin	6000 mg/dL
Gamma Globulin	6000 mg/dL

No cross reaction occurred with DKK-1 up to 10 µg/ml.

Remark

The data quoted in this instruction should be used for guidance only. It is recommended that each laboratory includes its own panel of control samples in the assay. In order to follow GLP guidelines, each laboratory should establish its own ranges for Sclerostin level.

TECO® Sclerostin HS ELISA

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature. Mix the samples well.
- Washing Buffer **2** : Dilute 1:50 with deionized or distilled water.

Prepare the required number of Assay Strips **1**

Pre-wash the plate by using 300 µl Wash Buffer for 2 min at 20-25°C

Pipette **25µl** Standards (Std. **A** – Std. **G**),Controls (**L** and **H**) and Samples
Add **50µl** Matrix Solution **4** (Multichannel pipette)
Add **50µl** Antibody Solution **5** (Multichannel pipette).

Incubate **4 h** at 20-25°C on a shaker (500 rpm)

Aspirate and wash **4 times** with 350 µl Wash Buffer, aspirate and tap the inverted wells on a clean dry absorbent surface

Pipette 100 µl TMB Substrate Solution **7**

Incubate 20 -30 min at 20-25°C in the dark on a shaker (500rpm)

Pipette 100 µl Stop Solution **8**

Read the Optical Density at 450nm and 405nm (reference filter between 590-650 nm).
Using 450nm: Calculate the sample values between 0 and 2ng/ml by using Std A - Std F
Using 405 nm: Calculate the values between 2 and 4 ng/ml by using Std A - Std G.
Use a 4-parameter curve fit for automatic data reduction

Please read Kit instruction before using the Quick Guide