Quantikine® ELISA

Human SOST Immunoassay

Catalog Number DSST00

For the quantitative determination of human SOST concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

SOST, also known as Sclerostin, is a Cerberus/DAN family member and is an important regulator of bone homeostasis (1). Cerberus/DAN proteins (Cerberus, DAN, Gremlin, PRDC, SOST, and Caronte) are secreted glycoproteins that function as BMP antagonists. While the overall sequence identity between members of the family is low, they share a cysteine-knot motif with conserved spacing of six cysteine residues. SOST is secreted as a monomer in contrast to many other cysteine-knot proteins which form disulfide-linked homodimers (2). Mature human SOST shares 89% and 92% amino acid (aa) sequence identity with mouse and rat SOST, respectively (3, 4). Alternative splicing of human SOST generates an isoform with a substitution for aa 64-73. Inactivating mutations in the SOST gene can cause sclerosteosis and van Buchem disease which are bone dysplasia disorders characterized by progressive skeletal overgrowth (3-5). SOST is expressed by terminally differentiated cells embedded in mineralized matrix including osteocytes, hypertrophic and prehypertrophic chondrocytes, and tooth cementocytes (6-9). SOST expression is induced by BMP-2, -4, and -6 (10, 11) and is inhibited by parathyroid hormone (PTH) (12, 13). Its downregulation in osteocytes by physical loading of bone contributes to the mechanical sensor function of osteocytes and the subsequent increase in bone growth (14).

SOST binds to BMP-2, -4, -5, -6, and -7 and inhibits the osteogenic differentiation induced by these BMPs (2, 6, 7). It inhibits canonical Wnt signaling by binding to LRP-5 and LRP-6 and inhibiting their association with Frizzled receptors (15, 16). SOST also modulates the ability of BMPR-IA signaling to interfere with canonical Wnt signaling (17). These interactions underlie the ability of SOST to inhibit BMP- and Wnt-induced bone formation *in vivo* (17, 18). SOST reduces the proliferation of mesenchymal stem cells (MSC) and induces MSC apoptosis (7, 19). It inhibits the differentiation of preosteoblastic cells and bone mineralization by osteoblasts (6, 7). In knockout mice that lack SOST expression, osteocyte and osteoblast apoptosis is inhibited, osteoblast activity is enhanced, and the mice are resistant to mechanical unloading-induced bone loss (20). SOST knockout mice also exhibit increased bone mineral density, bone volume, and bone strength throughout the skeleton (21). Mice treated with neutralizing anti-SOST antibodies likewise show increased bone formation and bone mineral density (22, 23). This treatment can reverse the bone loss and bone integrity decline that is otherwise seen in models of osteoporosis and chronic gut inflammation (22, 23).

Circulating levels of SOST are elevated in pathologies with bone involvement including low bone mineral density, Paget's disease, multiple myeloma, and prostate cancer with bone metastases (24-26). It is also elevated in advanced chronic kidney disease, alcoholic liver disease, type 2 diabetes, and patients with increased abdominal and gynoid fat (27-30). Serum SOST is negatively correlated with PTH and cortisol but positively correlated with free T3 and T4 thyroid hormones (31-33).

The Quantikine Human SOST Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human SOST in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human SOST and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human SOST showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring SOST.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human SOST has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SOST present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human SOST is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of SOST bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
SOST Microplate	894575	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human SOST.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
SOST Conjugate	894576	21 mL of a polyclonal antibody against human SOST conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
SOST Standard	894577	20 ng of recombinant human SOST in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives. *May contain crystals. Warm to room temperature and mix well to dissolve.*	
Calibrator Diluent RD5-20	895346	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human SOST Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not recommended for use in this assay.

REAGENT PREPARATION

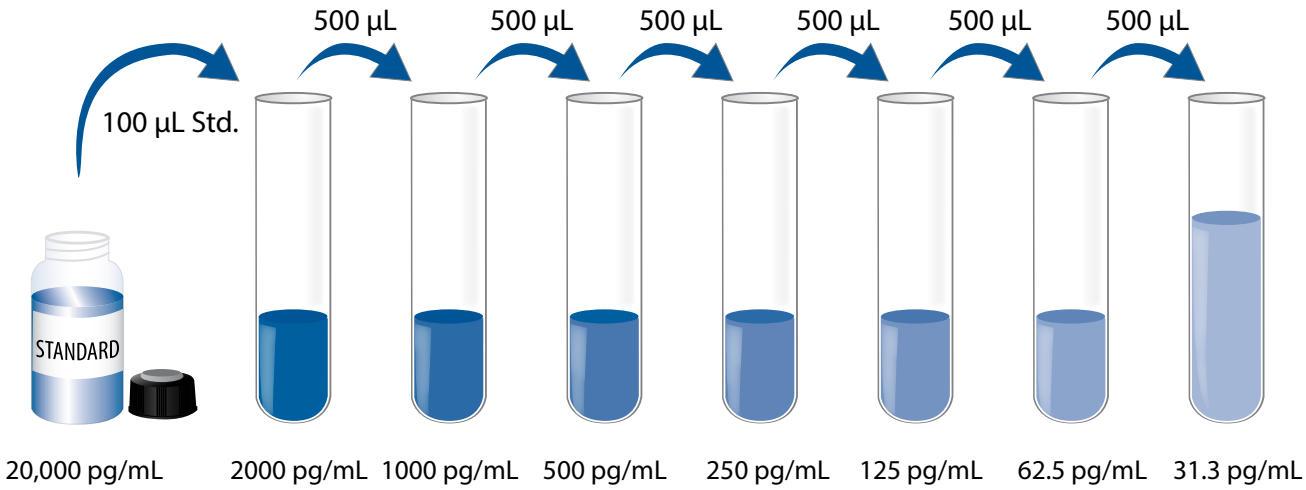
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

SOST Standard - Reconstitute the SOST Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-20 into the 2000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-20 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1X to each well. May contain crystals. *Warm to room temperature and mix well to dissolve before use.*
4. Add 50 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of SOST Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

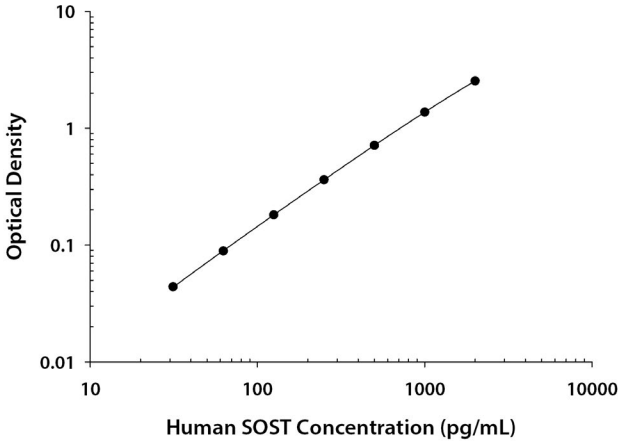
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human SOST concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
31.3	0.052 0.054	0.053	0.044
62.5	0.095 0.101	0.098	0.089
125	0.186 0.194	0.190	0.181
250	0.369 0.375	0.372	0.363
500	0.722 0.722	0.722	0.713
1000	1.369 1.396	1.383	1.374
2000	2.524 2.575	2.550	2.541

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	308	577	1208	315	591	1234
Standard deviation	6.03	10.5	25.1	25.9	63.8	118
CV (%)	2.0	1.8	2.1	8.2	10.8	9.6

RECOVERY

The recovery of human SOST spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	91-111%
Serum (n=4)	96	86-112%
EDTA plasma (n=4)	95	89-101%
Heparin plasma (n=4)	100	91-108%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human SOST were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	96	101	102	100
	Range (%)	94-98	100-102	100-104	99-101
1:4	Average % of Expected	94	100	102	99
	Range (%)	93-95	99-102	99-104	96-100
1:8	Average % of Expected	95	100	97	97
	Range (%)	93-97	98-103	94-101	92-99
1:16	Average % of Expected	88	96	94	92
	Range (%)	84-91	94-99	89-100	84-100

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human SOST ranged from 0.370-3.80 pg/mL. The mean MDD was 1.74 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human SOST manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human SOST in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	174	67.0-300	60.9
EDTA plasma (n=35)	426	200-712	127
Heparin plasma (n=35)	485	222-805	137

Cell Culture Supernates - Aliquots of cell culture supernates from 12 cells lines were assayed for human SOST. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human SOST.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human SOST control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

BMP-2
BMP-4
BMP-5
BMP-6
BMP-7
BMPR-IA
Cerberus
DAN
Gremlin
LRP-4
LRP-5
LRP-6

Recombinant mouse:

SOST

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