Product Manual

SCLEROSTIN

Catalog #: ENZ-KIT155

ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF SCLEROSTIN IN HUMAN SERUM, EDTA PLASMA OR HEPARIN PLASMA

1 x 96 wells

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

Canonical Wnt-signalling plays an important role in the regulation of bone homeostasis by promoting the development of osteoblasts. Negative regulators of the Wnt pathway are important new therapeutic targets for the treatment of diseases with enhanced bone resorption. One of these molecules is Sclerostin, a 22.5kD secreted glycoprotein, which acts by binding to the Wnt-co-receptor LRP5 thus preventing the binding of Wnt molecules. Sclerostin is nearly exclusively produced in osteocytes. Therefore it is considered a clinical marker which provides highest bone specificity.

Areas of Interest

- Osteoporosis
- Cancer induced bone diseases
- Rheumatoid arthritis
- Chronic inflammation
- Kidney diseases
- Therapy monitoring of anabolic treatment

MATERIALS SUPPLIED

CONT	KIT COMPONENTS	QUANTITY
PLATE	Polyclonal goat anti human Sclerostin antibody, pre-coated microtiter strips in a strip holder, in aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50ml
ASYBUF	Assay buffer, red cap, ready-to-use	1 x 20ml
AB	Monoclonal mouse anti human Sclerostin antibody – biotin labelled, green dye, green cap, ready-to-use	1 x 7ml
STD	Standard (0; 15; 30; 60; 120; 240pmol/l), white caps, lyophilised	6 vials
CTRL	Control, yellow cap, lyophilised (exact concentration on the label)	1 vial
CONJ	Conjugate, (streptavidin-HRPO), amber cap, ready-to-use	1 x 22ml
SUB	Substrate (TMB solution), amber bottle, blue cap, ready-to-use	1 x 22ml
STOP	Stop solution, white cap, ready-to-use	1 x 7ml

ADDITIONAL MATERIALS ADDED

- 2 self-adhesive plastic film
- Quality control protocol
- Protocol sheet
- Instruction manual for use

ADDITIONAL MATERIALS AND EQUIPMENT NEEDED

- Precision pipettes calibrated to deliver 50µl, 150µl, 200µl, 300µl, 500µl and disposable tips
- Distilled or deionized water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader for absorbance at 450nm (reference 630nm) Graph paper or software for calculation of results

REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until the expiry date stated on the label of each reagent.

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. We recommend performing plasma or serum 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 plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C, for long time storage at or below -70°C. All samples should undergo only 4 freeze-thaw cycles. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.

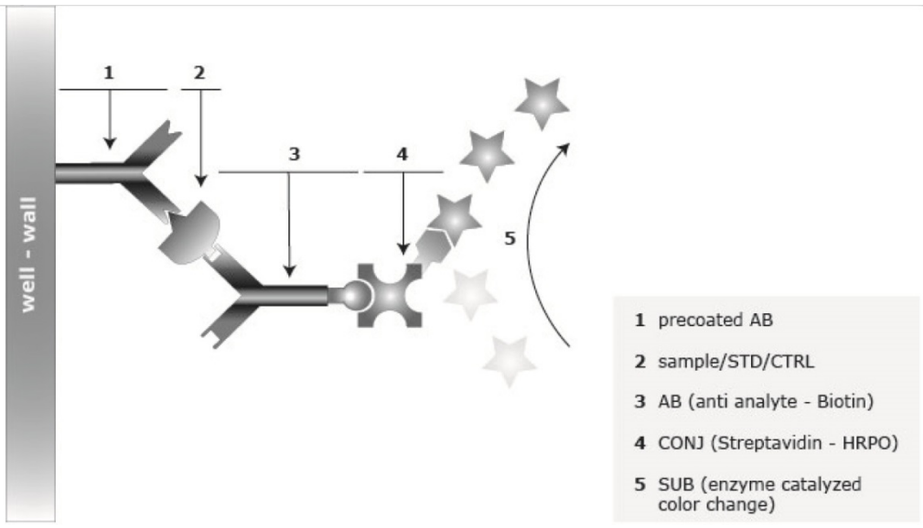
If samples read higher than the highest standard, we recommend diluting with ASYBUF provided in the kit and remeasuring the samples.

Reagent preparation:

WASHBUF (Wash buffer): Dilute the concentrate 1:20: e.g., 50ml WASHBUF + 950ml distilled water. Crystals in the buffer concentrate will dissolve at room temperature. The diluted buffer is stable at 4°C (2-8°C) until expiry date stated on the label. Use only diluted WASHBUF (Wash buffer) during the assay procedure.

STD (Standards) and CTRL (Control): Dissolve each in 400µl deionized or distilled water at room temperature for 15 min. Mix well (Vortex mixer). Reconstituted STD and CTRL are stable at -20°C until expiry date stated on the label. Avoid repeated freeze thaw cycles.

PRINCIPLE OF THE ASSAY



ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-24°C) before use in the assay.

Mark position for BLANK/STD (Standards)/ SAMPLE/CTRL (Control) on the supplied protocol sheet.

Take microtiter strips out of the aluminum bag, reserve a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the aluminum bag. Strips are stable until expiry date stated on the label.

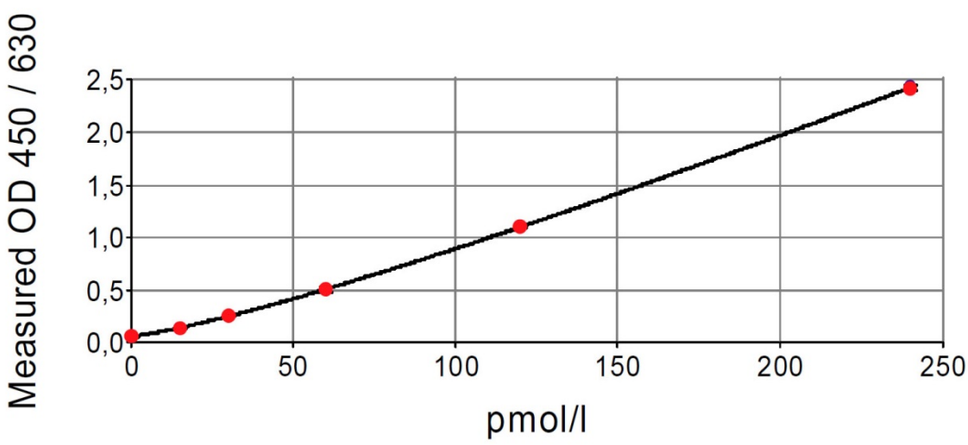
1. Add 150µl ASYBUF (red cap) into each well.
2. Add 20µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well.
3. Add 50µl AB (biotinylated anti Sclerostin antibody, green cap, green dye) into each well, swirl gently.
4. **Cover tightly and incubate overnight (18-24 h) at room temperature (18-24°C) in the dark. Attention: Incubation higher than room temperature reduces the top-OD.**
5. Aspirate and wash wells 5x with 300µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
6. Add 200µl CONJ (Conjugate, amber cap) into each well.
7. **Cover tightly and incubate for 1 hour at room temperature (18-24°C) in the dark.**
8. Aspirate and wash wells 5x with 300µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
9. Add 200µl SUB (Substrate, blue cap) into each well.
10. **Incubate for 30 min at room temperature (18-24°C) in the dark.**
11. Add 50µl STOP (Stop solution, white cap) into each well.
12. Measure absorbance immediately at 450nm with reference 630nm, if available.

CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450nm wavelength (correction wavelength 630nm). Subtract the blank OD from the values of STD, CTRL and sample. Construct the standard curve from the OD values of the STD. Use software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with a 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Respective dilution factors have to be considered.

If the OD of highest STD is outside the measuring range of photometer can be measured at 405nm (correction wavelength 630nm).

TYPICAL STANDARD-CURVE



The quality control protocol supplied with the kit shows the results of the final release QC for each kit at production date. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.00 or higher is obtained for the standard with the highest concentration and the control value is in range (target range see label).

ASSAY CHARACTERISTICS

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips			
Sample type:	Serum, plasma (EDTA, heparin), urine protocol available			
Standard range:	0 to 240pmol/l (6 standards and 1 control in human serum matrix)			
Conversion factor pg/ml to pmol/l:	1pg/ml = 0.044pmol/l (MW: 22.5kD)			
Sample volume:	20µl/well			
Sensitivity:	LOD: (0pmol/l + 3 SD): 3.2pmol/l; LLOQ: <7.5pmol/l			
Values of apparently healthy individuals:	Median Serum (n=411): 24.14pmol/l This value lies between calibration point 2 and 3 of the standard curve. It is recommended to establish the normal range for each laboratory.			
Incubation time , temperature:	18-24 h / 1 h / 30 min, room temperature (18-24°C)			
Cross reactivity:	The assay does not detect Wise (SOSTDC1) or Noggin. The assay does not cross react with rat or mouse Sclerostin.			
Precision:	Intra-assay (n=8) ≤ 7% , Inter-assay (n=6) ≤ 10%			
Spike/Recovery:	The mean recovery of recombinant Sclerostin in human serum samples (n=6) is 94%.			
Dilution linearity (average recovery of expected Sclerostin after a 1+1; 1+3; 1+7 dilution):	Dilution (serum samples):	1+1	1+3	1+7
	Endogenous Sclerostin	100%	113%	106%
	Recombinant Sclerostin	103%	93%	n.a.

PRECISION

Intra-Assay: 2 samples of known concentrations were tested 8 times by 1 operator within 1 kit lot.

Inter-Assay: 2 samples of known concentrations were tested 6 times within 3 different assay lots each by 2 different operators.

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	1.00
SD (pmol/l)	2.37	5.36	SD (pmol/l)	3.19	0.041
CV%	7%	5%	CV%	10%	3%

TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colorless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

PRECAUTIONS

- All test components of human source were tested with 3rd generation tests against HIV-Ab and HBsAg; and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.
- Liquid reagents contain ≤0.1% Proclin 300 as preservative.
- Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with the reagents by using gloves.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous. Irritations are possible – flush with water if contact occurs!

Handle with care

LITERATURE

1. Cejka D et al.: Renal elimination of sclerostin increases with declining kidney function. J Clin Endocrinol Metab (2014), 99: 248-255.
2. Bielesz BO et al.: Sclerostin Declines during Hemodialysis and Appears in Dialysate. Blood Purif 2014, 13;38(1): 30-36
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14. Modder UI et al.: Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res (2011), 26(2): 373-379.
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ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- Bring all reagents to room temperature (18-24°C).
- Prepare reagents and samples as instructed.
- Bring unused and prepared components to the storage temperature mentioned in the package insert.
- Take microtiter strips out of the aluminum bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- **Step 1**) Add 150µl ASYBUF (Assay buffer, red cap) into respective wells.
- **Step 2**) Add 20µl STD/ SAMPLE/ CTRL (Standard/ Sample/ Control) in duplicate into respective wells.
- **Step 3**) Add 50µl AB (biotinylated anti Sclerostin antibody, green cap) into each well, swirl gently.
- **Step 4**) **Cover tightly and incubate overnight (18-24h) at room temperature (18-24°C) in the dark. Attention: Incubation higher than room temperature reduces the top-OD.**
- **Step 5**) Aspirate and wash wells 5x with 300µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- **Step 6**) Add 200µl CONJ (Conjugate, brown cap) into each well.
- **Step 7**) **Cover tightly and incubate for 1 hour at room temperature (18-24°C) in the dark.**
- **Step 8**) Aspirate and wash wells 5x with 300µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- **Step 9**) Add 200µl SUB (Substrate, blue cap) into each well.
- **Step 10**) **Incubate for 30 min at room temperature (18-24°C) in the dark.**
- **Step 11**) Add 50µl STOP (Stop solution, white cap) into each well.
- **Step 12**) Read Optical Density immediately at 450nm with reference 630nm, if available.

NOTES

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