SCLEROSTIN

(EN) ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF SCLEROSTIN IN HUMAN SERUM, EDTA PLASMA OR HEPARIN PLASMA
CAT. NO. BI-20492 . 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 220524 (replacing 190109)

This kit was developed and manufactured by:
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1) 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 22.5 kD secreted glycoprotein, which acts by binding to the Wnt-coreceptor 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

2) CONTENT OF THE KIT

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, natural cap, concentrate 20x	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 20 ml
AB	Monoclonal mouse anti human Sclerostin antibody – biotin labelled, green dye, green cap, ready to use	1 x 7 ml
STD	Standard (0; 15; 30; 60; 120; 240 pmol/l), white caps, lyophilised	6 vials
CTRL	Control, yellow cap, lyophilised (exact concentration on the label)	1 vial
CONJ	Conjugate, (streptavidin-HRPO), amber bottle, amber cap	1 x 22 ml
SUB	Substrate (TMB solution), amber bottle, blue cap, ready to use	1 x 22 ml
STOP	Stop solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

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

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 20 µl, 50 µl, 150 µl, 200 µl, 400 µl, and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until 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 haemolysed 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 re-measuring the samples.

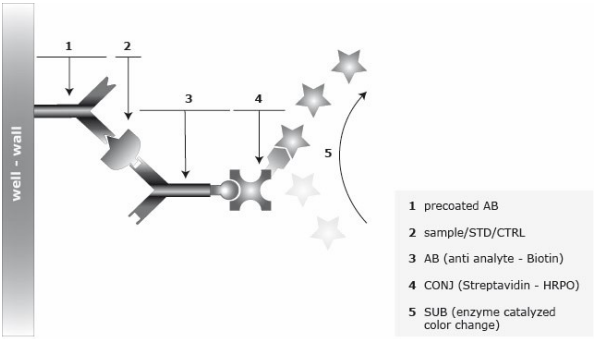
For further information on sample stability please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

Reagent preparation:

WASHBUF (Wash buffer): Dilute the concentrate 1:20: e.g. 50 ml WASHBUF + 950 ml 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 400 µl deionised 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.

6) PRINCIPLE OF THE ASSAY



7) ASSAY PROTOCOL

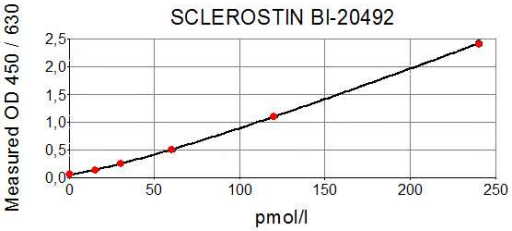
All reagents and samples must be at room temperature (18-24°C) before use in the assay.
Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

1) Add 150 µl ASYBUF (red cap) into each well.
2) Add 20 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well.
3) Add 50 µl 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 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
6) Add 200 µl 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 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
9) Add 200 µl SUB (Substrate, blue cap) into each well.
10) Incubate for 30 min at room temperature (18-24°C) in the dark.
11) Add 50 µl STOP (Stop solution, white cap) into each well.
12) Measure absorbance immediately at 450 nm with reference 630 nm, if available.

8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software. Obtain sample concentration from this standard curve. The assay was evaluated with 4PL algorithm. Different curve fitting methods need to be evaluated by the user.

Typical STD-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).

9) ASSAY CHARACTERISTICS

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips			
Sample type:	Serum, plasma (EDTA, heparin), urine protocol available			
Standard range:	0 to 240 pmol/l (6 standards and 1 control in human serum matrix)			
Conversion factor pg/ml to pmol/l:	1 pg/ml = 0.044 pmol/l (MW: 22.5 kD)			
Sample volume:	20 µl / well			
Sensitivity:	LOD: (0 pmol/l + 3 SD): 3.2 pmol/l; LLOQ: <7.5 pmol/l			
Values of apparently healthy individuals:	Median Serum (n=411): 24.14 pmol/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.

For details on validation data and assay characteristics please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

10) 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 by 2 different operators.

Intra-assay (n=8)	Sample 1	Sample 2
Mean (pmol/l)	33.6	118.8
SD (pmol/l)	2.37	5.36
CV (%)	7	5

Inter-assay (n=6)	Sample 1	Sample 2
Mean (pmol/l)	30.5	120.2
SD (pmol/l)	3.19	3.67
CV (%)	10	3

11) 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 colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

12) PRECAUTIONS

All test components of human source were tested against HIV-Ab, HCV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed of as if they were infectious.
Liquid reagents contain ≤0.1% Proclin 950 as preservative. Avoid contact with skin and mucous membrane. Proclin 950 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 reagents by using gloves.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous membrane. Irritations are possible – Flush with water if contact occurs!!

13) 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
3. Gaudio A et al.: The relationship between inhibitors of the Wnt signalling pathway (sclerostin and Dickkopf-1) and carotid intima-media thickness in postmenopausal women with type 2 diabetes mellitus. Diabetes and Vascular Disease Research (2014), 11: 48-52.
4. Kanbay M et al.: Serum sclerostin and adverse outcomes in nondialyzed chronic kidney disease patients. J Clin Endocrinol Metab 2014, 99(10): E1854-E1861
5. Ardawi MS et al.: Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes. Bone (2013), 56: 355-362.
6. Anastasilakis D et al.: Comparative Effect of Zoledronic Acid Versus Denosumab on Serum Sclerostin and Dickkopf-1 Levels of Naïve Postmenopausal Women With Low Bone Mass: A Randomized, Head-to-head Clinical Trial. J Clin Endocrinol Metab (2013), 98: 3206-3212.
7. Pelletier S et al.: The Relation between Renal Function and Serum Sclerostin in Adult Patients with CKD. Clin J Am Soc Nephrol (2013), 8: 819-823.
8. Ardawi MS et al.: High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: The center of excellence for osteoporosis research study. J Bone Miner Res (2012), 27(12): 2592-2602.
9. Saad CG et al.: Low sclerostin levels: a predictive marker of persistent inflammation in ankylosing spondylitis during anti-tumor necrosis factor therapy. Arthritis Res Ther, (2012), 14(5): R216.
10. Terpos E et al.: Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: reduction post-bortezomib monotherapy. Int J Cancer (2012), 131(6): 1466-1471.
11. Gennari L et al.: Circulating Sclerostin Levels and Bone Turnover in Type 1 and Type 2 Diabetes. J Clin Endocrinol Metab (2012), 97(5): 1737-1744.
12. Amrein K et al.: Sclerostin and Its Association with Physical Activity, Age, Gender, Body Composition, and Bone Mineral Content in Healthy Adults. J Clin Endocrinol Metab (2012), 97: 148-154.
13. García-Martín A et al.: Circulating Levels of Sclerostin Are Increased in Patients with Type 2 Diabetes Mellitus. J Clin Endocrinol Metab (2012), 97: 234-241.
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.
15. Gaudio A et al.: Increased Sclerostin Serum Levels Associated with Bone Formation and Resorption Markers in Patients with Immobilization-Induced Bone Loss. J Clin Endocrinol Metab (2010), 95: 2248-2253.

SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use)/ In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícky materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnicky materiál (určeno pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

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Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

BI-20492 SCLEROSTIN

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 aluminium bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- ❑ Step 1) Add 150 µl ASYBUF (Assay buffer, red cap) into respective wells.
- ❑ Step 2) Add 20 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells.
- ❑ Step 3) Add 50 µl AB (biotinylated anti Sclerostin antibody, green cap) into each well, swirl gently.
- ❑ **Step 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.**
- ❑ Step 5) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ❑ Step 6) Add 200 µl 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 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ❑ Step 9) Add 200 µl 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 50 µl STOP (Stop solution, white cap) into each well.
- ❑ Step 12) Read Optical Density immediately at 450 nm with reference 630 nm, if available.