BIOACTIVE SCLEROSTIN ELISA ASSAY

(EN) ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF BIOACTIVE SCLEROSTIN IN SERUM, EDTA PLASMA OR CITRATE PLASMA

CAT. NO. BI-20472 . 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

For the measurement of bioactive Sclerostin in cell culture supernatants or urine please visit our homepage www.bmgrp.com.

rev.no. 180124

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

1) INTRODUCTION

The human *bioactive Sclerostin* immunoassay is a 3.5 hour, 96-well, sandwich ELISA for the quantitative determination of bioactive Sclerostin in human serum, EDTA plasma, and citrate plasma. The assay employs human based serum standards to ensure biological reliable data. The antibody characterization of both antibodies utilized in this assay comprises epitope mapping, binding kinetics, and determination of antibody purity (for further information on antibody characterization please visit our website www.bmgrp.com, see Validation Data).

Sclerostin is a 22.5 kDa secreted glycoprotein that functions as a potent inhibitor of Wnt signaling. It acts by binding to the Wnt-coreceptor LRP5/6 thus inhibiting bone formation by regulating osteoblast function and promoting osteoblast apoptosis (1,2). 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 (3,4). Sclerostin is classically considered to be a monomeric protein, but data from *Hernandez and colleagues* (5) postulate that circulating sclerostin has a dimeric configuration. In addition, it is not yet well documented if also Sclerostin fragments circulate, but the comparison of different Sclerostin ELISAs suggest that fragments exist as well (6,7).

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

Sclerostin is nearly exclusively produced in osteocytes (8). Mutations in the Sclerostin (SOST) gene can cause sclerosteosis and van Buchem disease which are bone dysplasia disorders characterized by progressive skeletal overgrowth (9,10). Sclerostin levels are altered in response to hormonal stimuli or due to pathophysiological conditions. Sclerostin concentrations are increased in disorders such as hypoparathyroidism (11), Paget's disease (12), multiple myeloma (13) and in cancer induced bone diseases (12). Sclerostin levels are decreased in primary hyperparathyroidism (14), as well as by the mechanical stimulation of bone (15). Several studies have found a positive association between sclerostin and bone mineral density (16,17). Sclerostin levels in chronic kidney disease (CKD) patients are up to 4-fold increased compared to patients without CKD and increase with CKD stage and declining kidney function (18,19). In CKD patients, renal elimination of sclerostin increases with decreasing renal function (20). In dialysis patients, sclerostin is an independent predictor of bone loss (21). Numerous studies have shown that serum sclerostin levels are also associated with cardiovascular events (22,23). A monoclonal sclerostin antibody for the treatment of osteoporosis is currently undergoing clinical trials (24). For reviews please see references (25,26).

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	Recombinant human monoclonal 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 13 ml
STD	Standard 1-7 (0; 10; 20; 40; 80; 160; 320 pmol/l), white caps, lyophilised	7 vials
CTRL	Controls A+B, yellow caps, lyophilised (exact concentration on the label)	2 vials
CONJ	Conjugate (polyclonal goat anti human Sclerostin antibody)-HRPO, amber bottle, amber cap, ready to use	1 x 13 ml
SUB	Substrate (TMB solution), amber bottle, blue cap, ready to use	1 x 13 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, 100 µl, 250 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 (reference wavelength 630 nm)
- Graph paper or 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:
Serum, EDTA plasma, and citrate plasma are suitable for use in this assay. Don't change sample type during studies.

Collect venous blood samples by using standardized blood collection tubes. Perform serum or plasma (EDTA, citrate) separation by centrifugation according to supplier's instructions of the blood collection devices. The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. Do not freeze-thaw samples more than 4 times. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.
Samples with values above STD7 (320 pmol/l) can be diluted with ASYBUF (Assay buffer).

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

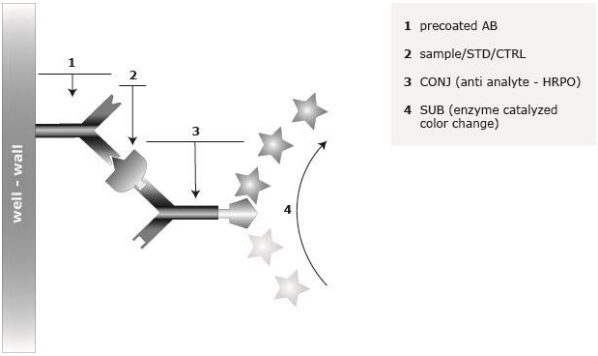
Reagent preparation:

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 undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Use only diluted WASHBUF when performing the assay.

STD (Standards) + CTRL (Controls): Pipette 250 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 15 min. Mix well. Reconstituted STDs and CTRLs are stable at -25°C or lower until expiry date stated on label. STDs and CTRLs are stable for at least 4 freeze-thaw cycles.

6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the quantitative determination of bioactive sclerostin in human serum and plasma samples (EDTA, citrate). In a first step, assay buffer is pipetted into the wells of the microtiter strips. Thereafter, STD/sample/CTRL are pipetted into the wells, which are pre-coated with the recombinant human monoclonal Sclerostin antibody. Any bioactive Sclerostin present in the STD/sample/CTRL binds to the pre-coated antibody in the well. After incubation, a washing step is applied where all non-specific unbound material is removed. In a next step, the conjugate (anti sclerostin-HRPO) is pipetted into the wells and reacts with bioactive Sclerostin present in the sample, forming a sandwich. After another washing step, the substrate (TMB Tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed colour change of the substrate is directly proportional to the amount of bioactive sclerostin. This colour change is detectable with a standard microtiter plate ELISA reader. A dose response curve of the absorbance (optical density, OD at 450 nm) versus standard concentration is generated, using the values obtained from the standards. The concentration of bioactive sclerostin in the sample is determined directly from the dose response curve.



Detailed information on the antibodies utilized in this ELISA can be found www.bmgrp.com, s. Validation Data.

7) ASSAY PROTOCOL

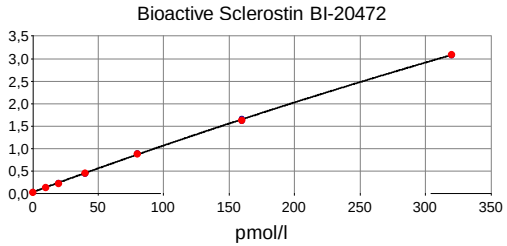
All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

1) Pipette 100 µl ASYBUF (Assay buffer, red cap) into each well.
2) Add 20 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well. Swirl gently.
3) Cover tightly and incubate for 2 hours at room temperature (18-26°C).
4) 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.
5) Add 100 µl CONJ (Conjugate, amber cap) into each well. Swirl gently.
6) Cover tightly and incubate for 1 hour at room temperature (18-26°C) in the dark.
7) 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.
8) Add 100 µl SUB (Substrate, blue cap) into each well. Swirl gently.
9) Incubate for 30 min at room temperature (18-26°C) in the dark.
10) Add 50 µl STOP (Stop solution, white cap) into each well. Swirl gently.
11) 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 (reference wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from the standard curve. The assay was evaluated with logit-log and 4PL algorithm curve fitting. 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, EDTA plasma, and citrate plasma (cell culture and urine protocol available)			
Standard range	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)			
Conversion factor	Sclerostin: 1 pg/ml = 0.046 pmol/l, 1 pmol/l = 21.793 pg/ml (MW: 21.8 kDa)			
Sample volume	20 µl / well			
Incubation time, temp.	2 h / 1 h / 30 min, room temperature			
Sensitivity	LOD: (0 pmol/l + 3 SD): 1.9 pmol/l; LLOQ: 1.3 pmol/l			
Specificity	This assay recognizes endogenous and recombinant human bioactive Sclerostin.			
Precision	Intra-assay (n=3) ≤ 1%, Inter-assay (n=7) ≤ 5%			
Spike/Recovery	Average % recovery spiked with 26 and 110 pmol/l, respectively	Serum (n=5): 93%, 86% EDTA plasma (n=5): 94%, 93% Citrate plasma (n=1): 104%, 99%		
Dilution linearity of recombinant Sclerostin	Average % of expected of dilution:	1+1	1+3	1+7
	Serum (n=6):	98	86	89
	EDTA plasma (n=6):	102	99	91
	Citrate plasma (n=1):	119	132	103
Dilution linearity of endogenous Sclerostin	Average % of expected of dilution:	1+1	1+3	1+7
	Serum (n=7):	100	103	106
	EDTA plasma (n=6):	105	108	123
	Citrate plasma (n=2):	91	91	103
Values of apparently healthy individuals	Median serum (n=32): 61.5 pmol/l Median EDTA plasma (n=24): 87 pmol/l Median citrate plasma (n=24): 61.5 pmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.			

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 export@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Intra-assay: 2 samples of known concentrations were tested 3 times with 1 assay lot by one 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
Mean (pmol/l)	19	153
SD (pmol/l)	0.3	1.0
CV (%)	1	1

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

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 300 as preservative. Avoid contact with skin and mucous membrane. 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 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

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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.
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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-20472 BIOACTIVE SCLEROSTIN

ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- ❑ Bring all reagents to room temperature (18-26°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) Pipette 100 µl ASYBUF (Assay buffer, red cap) into each well.
- ❑ Step 2) Add 20 µl STD/SAMPLE/CTRL (Standard/Sample/Control) into respective wells. Swirl gently.
- ❑ **Step 3) Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
- ❑ Step 4) 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 5) Add 100 µl CONJ (Conjugate, amber cap) into each well. Swirl gently.
- ❑ **Step 6) Cover tightly and incubate for 1 hour at room temperature (18-26°C) in the dark.**
- ❑ Step 7) 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 8) Add 100 µl SUB (Substrate, blue cap) into each well. Swirl gently.
- ❑ **Step 9) Incubate for 30 min at room temperature (18-26°C) in the dark.**
- ❑ Step 10) Add 50 µl STOP (Stop solution, white cap) into each well. Swirl gently.
- ❑ Step 12) Read Optical Density immediately at 450 nm with reference 630 nm, if available.