SOLUBLE SEMAPHORIN 4D

(EN) ELISA FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE SEMAPHORIN IN EDTA PLASMA, HEPARIN PLASMA, AND CITRATE PLASMA

Cat. No. BI-20405 . 12 x 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 180116

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CONTENT / INHALT

Detailed information on the assay characteristics including the validation data can be found on our website.

www.bmgrp.com

1) INTRODUCTION

Semaphorin 4D (SEMA4D or CD100) is a member of a family of transmembrane and secreted proteins that regulates key cellular functions and is involved in cell-cell communication (1-3). Most of the effects of SEMA4D is mediated by plexins (4, 5). SEMA4D participates in numerous physiological processes such as axon guidance, immune regulation, angiogenesis, tumor progression, and bone metabolism (6-9). Cleavage of SEMA4D near the cell membrane through matrix metalloproteinases leads to the biologically active soluble SEMA4D with a molecular weight of 120 kD consisting of 713 amino acids (3, 5, 10). SEMA4D has emerged to a novel therapeutic target in cancer and in bone diseases (11, 12). Semaphorin 4D is widely studied for its role in neural connectivity, vascularization, cell migration, the immune response, tumor progression, and bone remodeling.
This sSEMA4D ELISA utilizes two monoclonal anti-human Semaphorin 4D antibodies, both recognizing conformational epitopes on Semaphorin 4D. The epitopes have been mapped by overlapping cyclic peptides and shown to involve amino acids AA30-AA34 and amino acids AA238-AA241, respectively (for further information on antibody characterization please visit our website www.bmgrp.com: see Validation Data).

Areas of interest:

- Osteology
- Immunology
- Neurology
- Oncology

2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Monoclonal mouse anti-human Semaphorin 4D antibody pre-coated microtiter strips in a stripholder, packed in an aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
STD	Standards 1-7, (0; 62.5; 125; 250; 500; 1,000; 2,000 pmol/l), recombinant human soluble Semaphorin 4D in human plasma, white caps, lyophilised	7 vials
CTRL	Controls A and B, yellow caps, lyophilised, exact concentrations see labels	2 vials
ASYBUF	Assay buffer, red cap, ready to use	1 x 13 ml
CONJ	Conjugate (bivalent Fab bacterial alkaline phosphatase fusion antibody - HRP), 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 10 µl, 50 µl, 100 µl and 200 µl incl. disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

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

EDTA plasma, citrate plasma and heparin plasma are suitable for use in this assay. We do not recommend serum as sample matrix due to coagulation-induced sSEMA4D shedding (13). Do not change sample type during studies.

Sample preparation/dilution:

Collect venous blood samples by using standardized blood collection tubes for plasma. Perform plasma separation by centrifugation according to supplier's instructions of the blood collection devices and measure the acquired plasma samples as soon as possible. For longer storage aliquot samples and store at -25°C or lower. Samples are stable for 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.
Samples with values above highest STD 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.

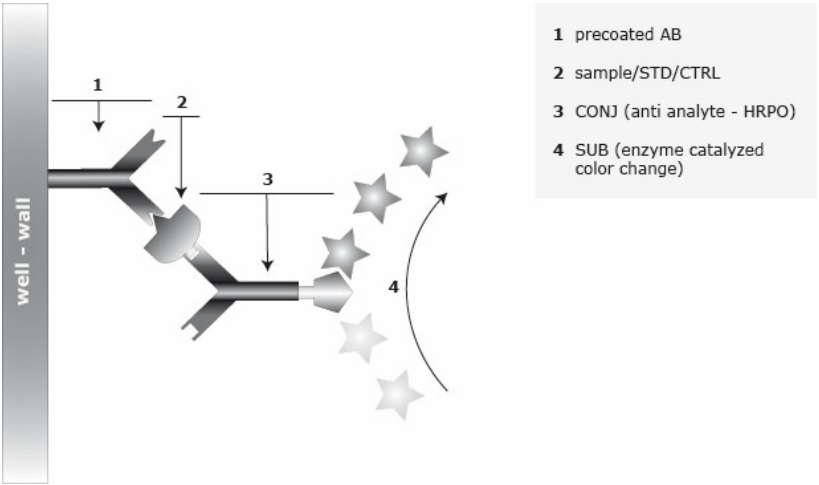
Reconstitution/Handling:

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). Only use diluted WASHBUF when performing the assay.

STD (Standards) + CTRL (Controls): Pipette 200 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 15 min. Vortex gently. Make sure that lyophilisate is completely dissolved. The exact concentration is printed on the label. Reconstituted STDs and CTRLs are stable at -25°C or lower until expiry date stated on the label. STDs and CTRLs are stable for 4 freeze-thaw cycles.

6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the direct determination of soluble Semaphorin 4D in human plasma samples. In a first step, STD/sample/CTRL are pipetted into the wells of the microtiter strips, which are pre-coated with anti Semaphorin 4D antibody. Semaphorin 4D present in the STD/sample/CTRL binds to the pre-coated antibody in the well. In the washing step all non-specific unbound material is removed. In a next step, the conjugate (bivalent Fab bacterial alkaline phosphatase fusion antibody-HRP) is pipetted into the wells and reacts with the soluble Semaphorin 4D 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 Semaphorin 4D. 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 Semaphorin 4D in the sample is determined directly from the dose response curve.



7) ASSAY PROTOCOL

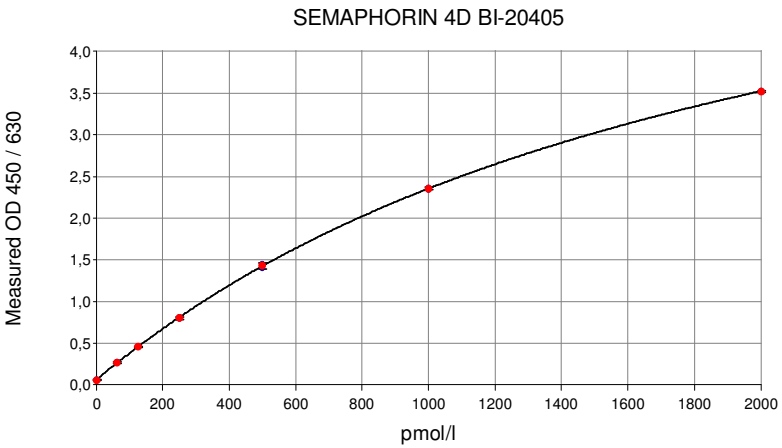
All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are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

1. Pipette 100 µl ASYBUF (Assay buffer, red cap) into each well.
2. Add 10 µl STD/CTRL/SAMPLE (Standard/Control/Sample) in duplicate into respective wells, swirl gently.
3. Cover tightly and incubate for 3 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 (correction 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 this 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. Respective dilution factors have to be considered when calculating the final concentration of the sample.

Example typical STD-curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot. 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 1.50 or more is obtained for the STD with the highest concentration and the values of the CTRLs are in range (target ranges see labels).

9) ASSAY CHARACTERISTICS

Method:	Sandwich ELISA, HRP/TMB, 12x8-well strips		
Sample type:	EDTA plasma, citrate plasma and heparin plasma		
Standard range:	0 to 2,000 pmol/l (0 / 62.5 / 125 / 250 / 500 / 1,000 / 2,000) 7 standards and 2 controls in a human plasma matrix.		
Conversion factor:	soluble Semaphorin 4D: 1 pg/ml = 0.0127 pmol/l; 1 pmol/l=78.9 pg/ml (MW: 78.9 kDa)		
Sample volume:	10 µl / well		
Incubation time:	3 h / 1 h / 30 min		
Sensitivity:	LOD: (0 pmol/l + 3 SD): 12 pmol/l; LLOQ: 31 pmol/l		
Specificity:	This assay recognizes endogenous and recombinant human soluble Semaphorin 4D.		
Precision:	Intra-assay (n=5) ≤ 8% , Inter-assay (n=11) ≤ 11%		
Spike/Recovery (200 + 1,000 pmol/l Semaphorin 4D)	Average % recovery	200 pmol/l	1,000 pmol/l
	EDTA plasma (n=6):	116	92
	Heparin plasma (n=2)	94	109
	Citrate plasma (n=2)	79	83
Dilution of endogenous soluble Semaphorin 4D:	Average % of expected of dilution	1+1	1+3
	EDTA plasma (n=4)	106	92
	Citrate plasma (n=2)	103	93
	Heparin plasma (n=2)	111	106
Values from apparently healthy individuals:	Median EDTA plasma (n=44): 245 pmol/l Median citrate plasma (n=43): 192 pmol/l Median heparin plasma (n=28): 201 pmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.		

For further information on 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 concentration were tested 5 times within 1 kit lot by 1 operator.
Inter-assay: 2 samples of known concentration were tested 11 times within 2 different kit lots by 4 different operators.

Intra-assay (n=5)	Sample 1	Sample 2
Mean (pmol/l)	126	1,003
SD (pmol/l)	10.4	63.8
CV (%)	8	6

Inter-assay (n= 11)	Sample 1	Sample 2
Mean (pmol/l)	134	1,012
SD (pmol/l)	14.5	55.1
CV (%)	11	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 as if they were infectious.
All 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.
- Wear gloves, glasses, and lab coat while performing this assay.
- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs!

13) LITERATURE

1. Semaphorins command cells to move. Kruger RP et al., Nature Rev Mol Cell Biol, 2005; 6:789-800.
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3. Biology and function of neuroimmune semaphorins 4A and 4D. Nkyimbeng-Takwi EH and Chapoval SP, Immunol Res, 2011; 50 (1):10-21.
4. Structural basis of semaphorin-plexin signalling B.J.C. Janssen BJC et al., Nature, 2010; 467:1118-1122.
5. Semaphorins and their receptors in immune cell interactions. Suzuki K et al., Nature Immunology, 2007; 9:17-23.
6. Sema4D induces angiogenesis through Met recruitment by Plexin B1. Conrotto P et al., Blood, 2005; 105:4321-4329.
7. Diverse roles for semaphorin-plexin signaling in the immune system. Takamatsu H et al., Trends Immunol, 2012; 33(3):127-135.
8. Bone cell communication factors and Semaphorins. Negishi-Koga T and Takayanagi H, Bonekey Rep, 2012; 1:183.
9. Suppression of bone formation by osteoclastic expression of semaphorin 4D. Negishi-Koga T et al., Nat Med, 2011; 17(11):1473-1480.
10. Soluble SEMA4D/CD100: A novel immunoregulator in infectious and inflammatory diseases. Maleki KT et al., Clinical Immunology, 2016; 163:52-59.
11. Anabolic bone formation via a site specific bone targeting delivery system by interfering with semaphorin 4D expression. Zhang Y et al., J Bone Miner Res, 2015; 30(2): 286-296.
12. Generation and preclinical characterization of an antibody specific for SEMA4D. Fisher TL et al., Mabs, 2016; 8(1):150-162.
13. Coagulation-induced elevated sSEMA 4D concentrations in human serum versus plasma measured by sandwich ELISA. Laber et al., 2018; submitted.

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

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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-20405 soluble SEMAPHORIN 4D ELISA
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) into all wells.
- ❑ Step 2) Pipette 10 µl STD/SAMPLE/CTRL (standard/sample/control) into respective wells, swirl gently.
- ❑ **Step 3) Cover tightly and incubate 3 hours at room temperature (18-26°C).**
- ❑ Step 4) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by strongly tapping plate against paper towel after the last wash.
- ❑ 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 with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by strongly tapping plate against paper towel after the last wash.
- ❑ Step 8) Add 100 µl SUB (Substrate, blue cap) into each well, swirl gently.
- ❑ **Step 9) Incubate for 30 minutes 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 11) Read Optical Density at 450 nm with reference 630 nm, if available.