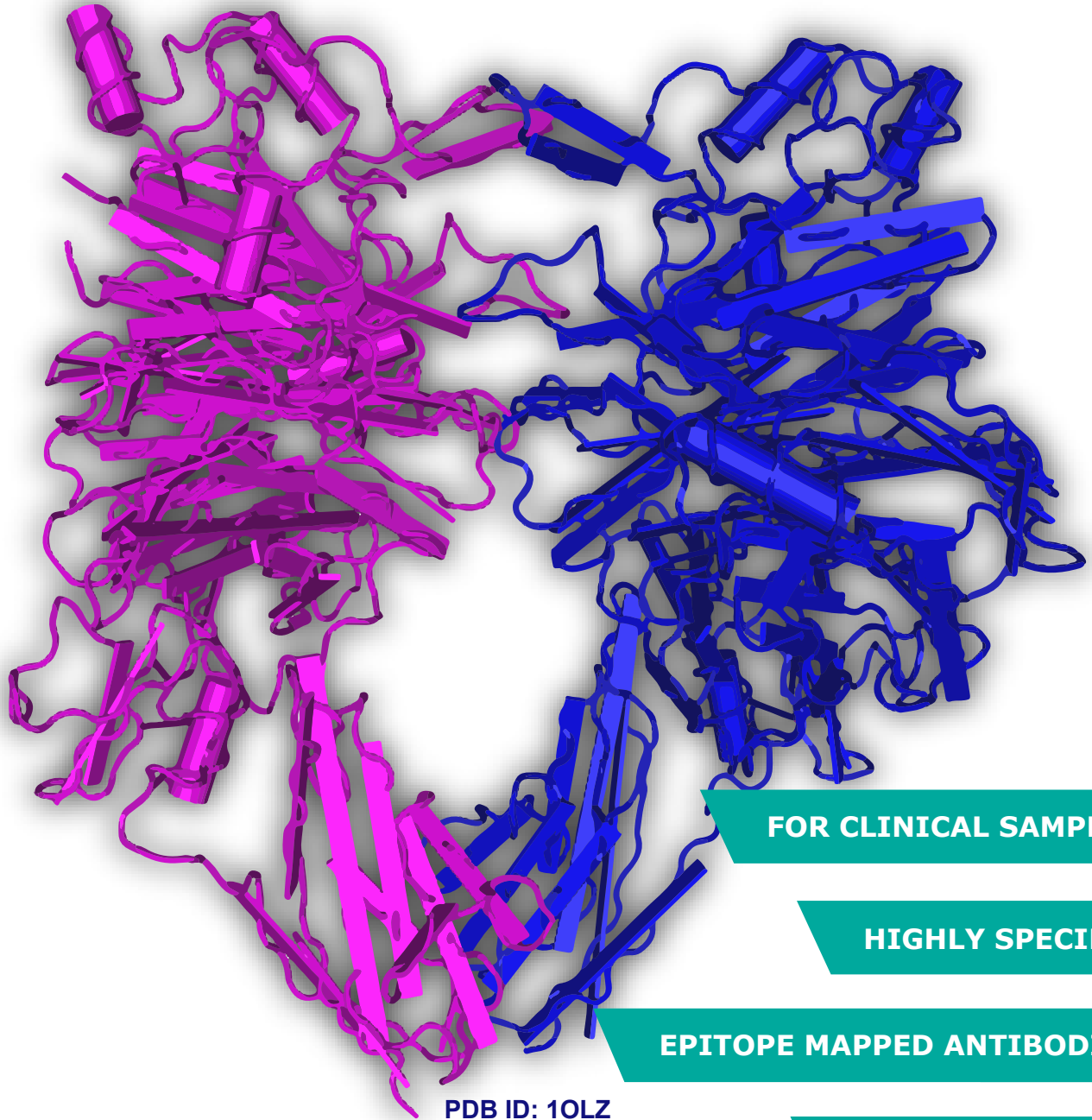


SEMAPHORIN 4D ELISA



PDB ID: 1OLZ
(ref.12)

FOR CLINICAL SAMPLES

HIGHLY SPECIFIC

EPITOPE MAPPED ANTIBODIES

FULLY VALIDATED

Setting the **standard**
for **clinical** research.



Background

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 (1,2). Most of the effects of SEMA4D is mediated by plexins (3,4). SEMA4D participates in numerous physiological processes such as axon guidance, immune regulation, angiogenesis, tumor progression, and bone metabolism (4-7). Cleavage of SEMA4D near the cell membrane through matrix metalloproteinases leads to the biologically active soluble SEMA4D (sSEMA4D) with a molecular weight of 120 kD consisting of 713 amino acids (2,3,7). SEMA4D has emerged to a novel therapeutic target in cancer and in bone diseases (8,9).

Areas of Interest

Osteology, Immunology, Neurology, Oncology

Semaphorin 4D is widely studied for its role in neural connectivity, vascularization, cell migration, the immune response, tumor progression, and bone remodeling.

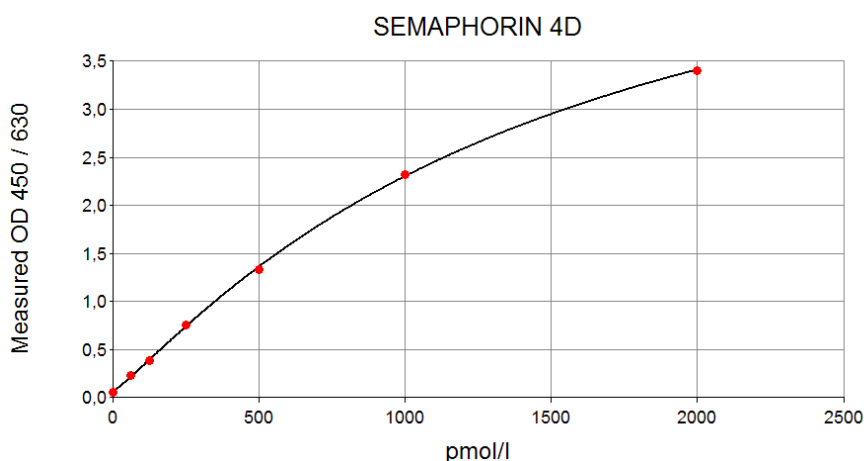
Features and Benefits

- LOW SAMPLE VOLUME – only 10µl / well required
- FULLY VALIDATED – according to ICH, EMEA and FDA guidelines
- CONVENIENT PROTOCOL – ready to use reagents included
- PROPRIETARY PRODUCT – in-house R&D and production
- GUARANTEED PERFORMANCE – rigorous validation and QC
- HIGHLY SPECIFIC – characterized antibodies and reagents

Assay Characteristics

- Method: Sandwich ELISA, HRP/TMB, 12x8-well strips
- Sample type: EDTA plasma, citrate plasma and heparin plasma
- Sample size: 10 µl / well
- Standard range: 0 to 2,000 pmol/l (7 standards and 2 controls in a human plasma matrix)
- Sensitivity: LOD (0 pmol/l + 3 SD): 12 pmol/l; LLOQ: 31 pmol/l
- Incubation: 3 h / 1 h / 30 min
- Unit conversion: 1 pmol/l=78.9 pg/ml (MW: 78.9 kDa)

Typical Standard Curve



soluble SEMAPHORIN 4D ELISA (Cat.No. BI-20405)

Specificity

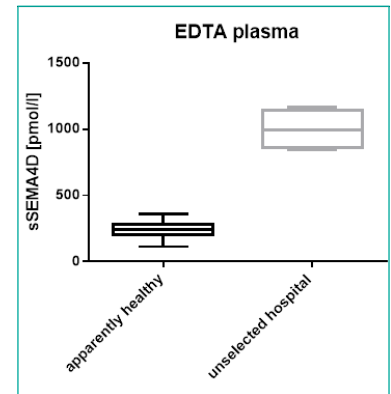
The assay is optimized to detect soluble human Semaphorin 4D in human plasma.

The soluble Semaphorin 4D 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: www.bmgrp.com, Validation Data.

sSEMA4D Values in Human Plasma Samples

sSEMA4D	EDTA plasma [pmol/l]	
	unspecific hospital panel (n=4)	apparently healthy panel (n=44)
Mean	997	239
Median	991	245
Minimum	841	113
Maximum	1,165	357



Spike/Recovery

Matrix	Mean S/R [%]	
	+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 Linearity

Matrix	Mean R of dilution steps [%]		
	1+1	1+3	1+7
EDTA plasma (n=4)	106	92	99
Citrate plasma (n=2)	110	109	121
Heparin plasma (n=2)	103	93	133

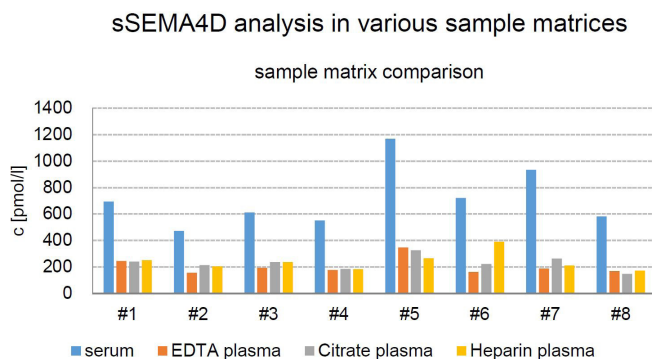
Precision

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

Why we don't recommend serum as matrix to measure soluble Semaphorin 4D?

We analyzed soluble SEMA4D in both serum and plasma samples. Based on our results we do not recommend the use of serum as matrix for sSEMA4D analysis. A comparison between sSEMA4D levels in serum and plasma resulted in significantly elevated sSEMA4D levels in serum. This can be explained that plasma anticoagulants prohibit coagulation-induced platelet activation that might lead to sSEMA4D shedding. Zhu and colleagues demonstrated that blood coagulation-related platelet activation, e.g. due to vascular injury in the course of sample collection, leads to increased sSEMA4D surface expression, followed by shedding into the circulation (10). We could demonstrate that plasma is free of shed sSEMA4D and is a suitable matrix for reproducible sSEMA4D quantification (11).



Related Biomedica Products

- Osteoprotegerin ELISA (cat.no. BI-20403)
- Neuropilin-1 ELISA (cat.no. BI-20409)
- DKK-1 ELISA (cat.no. BI-20413)
- Periostin ELISA (cat.no. BI-20433)
- free soluble RANKL ELISA (cat.no. BI-20462)
- bioactive Sclerostin ELISA (cat.no. BI-20472)
- Sclerostin ELISA (cat.no. BI-20492)
- FGF23 (C-terminal) ELISA (cat.no. BI-20702)
- Endostatin ELISA (cat.no. BI-20742)
- osteomiR™ miRNA Biomarkers (cat.no. TW-KW-011-OT)

Literature

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4. Diverse roles for semaphorin-plexin signaling in the immune system. Takamatsu H et al., *Trends Immunol*, 2012; 33(3):127-35.
5. Bone cell communication factors and Semaphorins. Negishi-Koga T, Takayanagi H, *Bonekey Rep*, 2012; 1:183.
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7. Soluble SEMA4D/CD100: A novel immunoregulator in infectious and inflammatory diseases. Maleki KT et al., *Clinical Immunology*, 2016; 163: 52-59.
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11. Analytical performance evaluation of a high-sensitivity enzyme immunoassay for soluble human semaphorin 4D in plasma. Laber et al., 2018; submitted.
12. The Ligand-Binding Face of the Semaphorins Revealed by the High-Resolution Crystal Structure of Sema4D. Love CA et al., *Nat Struct Mol Biol*, 2003; 10: 843