

bioactive Sclerostin ELISA, BI-20472

Protocol for cell culture measurements

MEASUREMENT of bioactive Sclerostin in CELL CULTURE SUPERNATANTS – performance check

Note: the experiments performed to measure bioactive Sclerostin in cell culture supernatants are not a full validation but are merely a performance check.

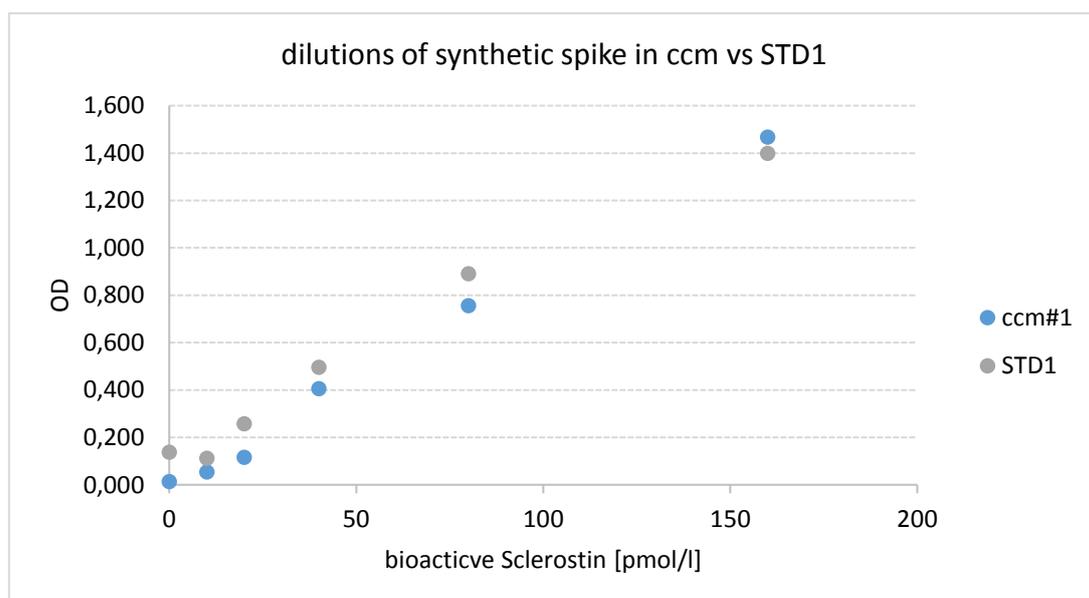
Cell culture medium (ccm: RPMI1640 containing 10% fetal calf serum) was tested undiluted and spiked with a final concentration of 160 pmol/l recombinant human bioactive Sclerostin. The spiked solution was diluted 1+1, 1+3, 1+7 and 1+15 with the cell culture medium.

As a comparison, the spike recovery and dilution linearity of the standard matrix (=STD1) and the dilutions with assay buffer is shown.

OD values of spiked and diluted cell culture medium sample and standard matrix (STD1):

Dil medium	Sample ID	OD					
		Reference	+ 160 pmol/l	1+1	1+3	1+7	1+15
ccm	ccm	0.014	1.468	0.756	0.406	0.116	0.054
ASYBUF	STD1	0.138	1.400	0.892	0.496	0.258	0.113

Graph showing dilution of cell culture medium (ccm) and a comparison to the Standard (STD1), both spiked with the same amount of synthetic bioactive Sclerostin (160 pmol/l).



Suggested protocol for the measurement of human bioactive Sclerostin in cell culture supernatants

Preparation of a cell culture medium (ccm) based standard curve:

Reconstitute STD7 in 250 µl deionized water. Leave at room temperature (18-26°C) for 15 min and mix well prior to making dilutions.

Use polypropylene tubes.

For the preparation of the cell culture based standards *always* use the identical cell culture medium in which the samples are based on.

- Mark tubes e.g. CC STD6, CC STD 5 ... CC STD1.
- Prepare a two-fold serial dilution to obtain STD6 to STD2.

e.g.:

Dispense 100 µl cell culture medium into vials labelled with CC STD6 to CC STD1.

Pipette 100 µl of STD 7 into tube marked as CC STD6. Mix thoroughly.

Transfer 100 µl of CC STD6 into vial marked as CC STD5. Mix thoroughly. Continue in the same fashion to obtain CC STD4 to CC STD2.

- ccm serves as the zero standard (=CC STD1, 0 pmol/l).

Attention: Concentrations defined for CTRL A and B are only valid for measuring bioactive Sclerostin in human serum or plasma. The controls cannot be used for cell culture measurements.