

Total soluble Neuropilin-1 ELISA, BI-20409

Protocol for cell culture measurements

MEASUREMENT of total soluble Neuropilin-1 in CELL CULTURE SUPERNATANTS

Performance check

Note: the experiments performed to measure total soluble Neuropilin-1 in cell culture supernatants are not a full validation but are merely a performance check.

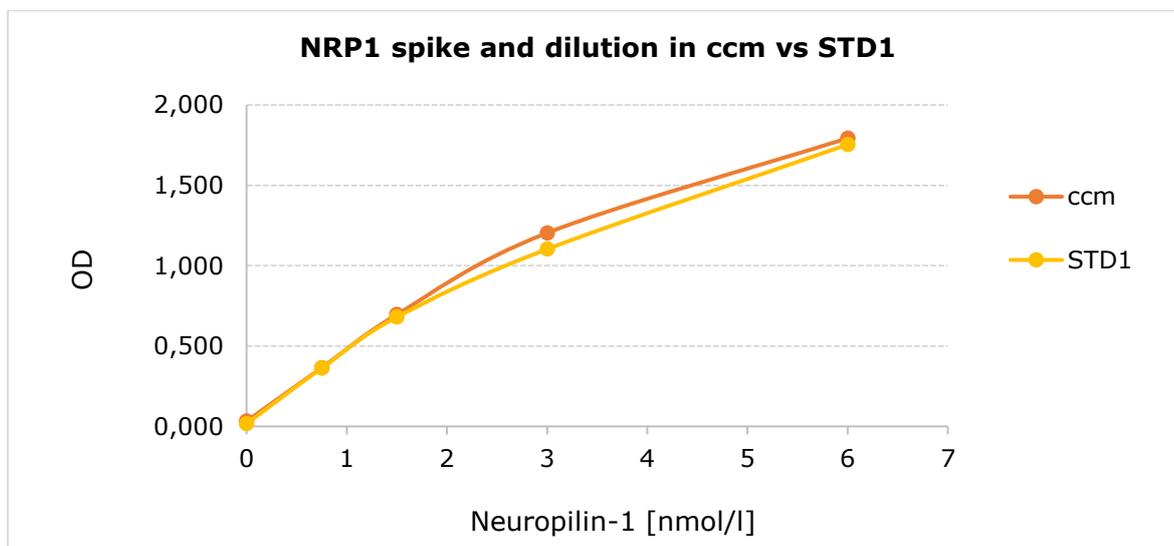
Cell culture medium (ccm: RPMI1640 containing 10% fetal calf serum) was tested undiluted and spiked by adding STD7 (provided in the kit, final concentration 6 nmol/l human recombinant Neuropilin-1) directly to cell culture medium (ratio 1+1). The spiked solution was diluted 1+1, 1+3 and 1+7 with the cell culture medium.

As a comparison, the spike recovery and dilution linearity of the standard matrix (=STD1, containing 0 nmol/l human recombinant Neuropilin-1) and the dilutions with cell culture medium is shown.

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

Dilution medium	Sample ID	Neuropilin-1 [nmol/l]				
		Reference	+ 6 nmol/l	1+1	1+3	1+7
ccm	ccm	0	6.0	3.2	1.6	0.8
ccm	STD1	0	5.8	2.8	1.5	0.7

Graph showing dilution of cell culture medium (ccm) and a comparison to the standard (STD1, containing 0 nmol/l human NRP1). Both ccm and STD1 were spiked with the same amount of human Neuropilin-1 (6 nmol/l).



Protocol for the measurement of human Neuropilin-1 in cell culture supernatants

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

Reconstitute STD7 in 200 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 medium 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.

- Cell culture medium serves as the zero standard (=CC STD1, 0 nmol/l).

NOTE: after preparation of the cell culture medium (ccm) based standard curve, proceed with the protocol as indicated in the instructions for use. ALL samples including the ccm based standards must be treated with GuHCl before assaying.

Attention: Concentrations defined for CTRL A and B are only valid for measuring total soluble Neuropilin-1 in human serum or plasma. The controls cannot be used for cell culture measurements.

Suggested protocol for the measurement of human Neuropilin-1 in cell culture supernatants (cc)

Follow standard protocol as indicated in the package insert:

In pre-dilution plate:

Use 10 µl cc sample for GuHCl treatment.

In pre-coated plate:

Transfer 50 µl pre-treated cc sample from pre-dilution plate into respective wells. Swirl gently.

For the transfer into the coated plate it is recommended to use a multichannel pipette. Transfer should be performed as soon as possible.

If required, dilute samples 1+1 with ccm (cell culture medium) prior to GuHCl treatment.