HUMAN CDNF QUANTIFICATION KIT INSTRUCTION MANUAL

APPLICATION

The human CDNF Quantification kit provides a rapid and easy method for the quantitative determination of human CDNF in cell culture supernatant, serum and plasma. The kit includes ready-to-use reagents necessary to analyse up to 88 samples in 2 hours.

PRINCIPLE OF THE ASSAY

The human CDNF test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with human CDNF-specific monoclonal capture antibodies. Samples and standards are pipetted into microwells and human CDNF molecules present in the sample are bound by the capture antibodies. After incubation, unbound material is removed by washing the wells. Then, horseradish peroxidase (HRP) conjugated human CDNF-specific monoclonal detection antibodies bind to a different epitope of human CDNF molecules. After washing, the ready to use HRP substrate (TMB) is added to wells. The intensity of the colour produced is directly proportional to the amount of human CDNF in the sample. Colour development is then stopped by the addition of stop solution. Absorbance is measured at 450 nm.

SENSITIVITY

The detection range is from 15 pg/mL to 960 pg/mL.
The detection limit is 2 pg/mL to 8 pg/mL, defined by the minimum human CDNF concentration deviating by 2 standard deviations (2SD) from that of the standard A. The test was performed by using 16 replicate determinations of standard A (blank) and standard B.

STORAGE CONDITIONS

The kit should be stored at +2...+6°C. Unopened, the kit will remain stable until the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the individual component. After opening, the components should be used within 8 weeks (microwell plate desiccation recommended).

KIT CONTENTS

- Pre-coated microwell plates: 96 microwells coated with anti-human CDNF mouse monoclonal antibodies.
- Human CDNF sample diluent, 25 mL, pink solution (PBS pH7.4, BPLA, detergent and preservative)
- Human CDNF standards A-H, 1 mL, pink solution (0-15-30-60-120-240-480-960 pg/mL)
- Human CDNF enzyme conjugate, 12 mL, blue solution. HRP-conjugated mouse monoclonal antibody in a buffered solution containing BPLA, detergent and preservative.
- Wash concentrate, 50 mL (PBS pH 7.4 and detergent)
- Substrate solution (TMB), 12 mL
- Stop solution (0.5 M H_2SO_4), 12 mL

MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and tips (100-500 µl)
- ELISA plate washer
- Microplate reader (450 nm)
- Lid or sealing tape for microwell plate
- Microwell plate shaker

PREPARATION OF SAMPLES

Dilute the samples in sample diluent.

ASSAY PROCEDURE

Allow all reagents to reach room temperature (RT) (20-22° C) before use (30 minutes).
Take the required number of microplate strips and place the remaining strips back into the vacuum bag. Close the bag tightly.

STEP 1	Dilute 50 mL of wash concentrate with 450 mL of distilled water to prepare washing solution.
STEP 2	Perform dilutions of each sample in sample diluent (pink).
STEP 3	Add 100 µL of samples and standards (pink) into appropriate wells in duplicate.
STEP 4	Incubate the covered microplate for 1 hr at RT on a microwell plate shaker (300 rpm).
STEP 5	Discard the solution and wash the wells 4 times with 300 µL of washing solution.
STEP 6	Add 100 µL of enzyme conjugate (blue) into each well.
STEP 7	Incubate the covered microplate for 30 min at RT on a microwell plate shaker (300 rpm).
STEP 8	Discard the solution and wash the wells 4 times with 300 µL of washing solution.
STEP 9	Add 100 µL of substrate solution into each well.
STEP 10	Incubate the covered microplate for 10 - 25 minutes (the precise incubation time comes with the kit) at RT on a microwell plate shaker (300 rpm).
STEP 11	Stop the reaction by adding 50 µl of STOP solution into each well in the same order and time as for TMB distribution.
STEP 12	Read the absorbance at 450 nm immediately.

CALCULATION OF RESULTS

Standard curve: Calculate the mean absorbance for each standard. Subtract the blank value (standard A) from the mean absorbances. Plot the value (absorbance) of each standard on a log-log scale. The use of software to generate a cubic spline fit curve is recommended
The human CDNF concentration in the sample can be calculated by interpolation between standard points on the curve.

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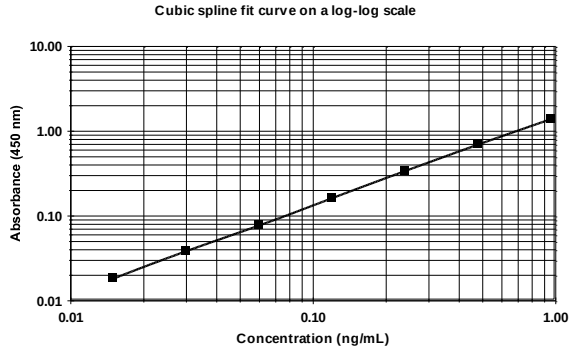
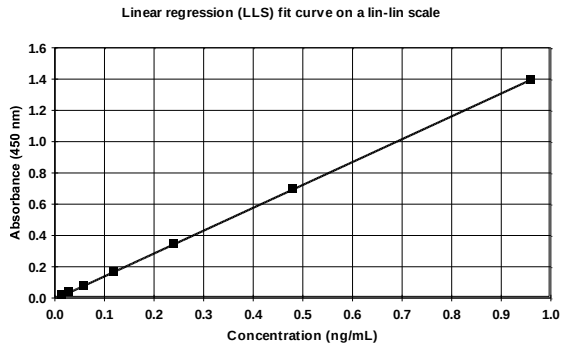
When generating a linear regression fit curve instead of a cubic spline fit curve only minor differences occur in human CDNF concentration calculation.

Validation of the assay: The mean absorbance of the Standard A (blank) should be below 0.1 AU (absorbance unit). The mean absorbance of the Standard H is usually above 1.0 AU.

TYPICAL DATA

These standard curves are shown as an example of a typical assay (Not to be used for calculation of actual test results).





PRECISION

Intra-assay precision:

Sample	Number of measures	Mean (pg/mL)	CV%
1	15	677	3.7
2	15	272	2.8
3	15	167	2.5

Inter-assay precision:

Sample	Number of assays	Mean (pg/mL)	CV%
1	2	19	8.1
2	2	155	16.0
3	2	439	20.8

LINEARITY (DILUTION TEST)

Three samples (plasma or serum) were diluted with sample diluent. The concentration of human CDNF in each diluted sample was measured. The results are shown as a change in percentage from the lowest dilution (corrected with the dilution factor).

Sample	Dilution	Conc. (pg\mL)	%
Serum	1:10	870	100
	1:20	950	109
	1:40	950	109
Plasma #1	1:10	1420	100
	1:20	1270	89
	1:40	1280	90
Plasma #2	1:03	120	100
	1:06	100	83
	1:12	130	108

RECOVERY

Human CDNF standards of 120, 480 and 960 pg/mL were added to equal volumes of three samples (serum or plasma) containing a low (50 pg/mL), a medium (220 pg/mL) and a high (730 pg/mL) concentration of human CDNF. The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (pg/mL)	Expected conc. (pg/mL)	Obtained conc. (pg/mL)	Recovery %
Low	0		50	100
	120	85	70	82
	480	290	260	90
	960	510	510	100
Medium	0		220	100
	120	170	120	71
	480	350	330	94
	960	590	620	105
High	0		730	100
	120	430	310	72
	480	610	600	98
	960	850	880	103

TECHNICAL ASSISTANCE

Please refer any technical questions to technical.support@icosagen.com.