HUMAN NEURTURIN QUANTIFICATION KIT INSTRUCTION MANUAL

APPLICATION

The human Neurturin (hNRTN) Quantification kit provides a rapid and easy method for the quantitative determination of hNRTN in cell culture supernatant, serum and plasma. The kit includes ready-to-use reagents necessary to analyse up to 89 samples in 2 hours.

PRINCIPLE OF THE ASSAY

The hNRTN test is based on the quantitative sandwich enzyme immunoassay technique. Microtiter wells are pre-coated with hNRTN-specific polyclonal capture antibodies. Samples and standards are pipetted into microwells and hNRTN 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 hNRTN-specific polyclonal detection antibodies bind to the same epitopes on another molecule of dimeric hNRTN. After washing, the ready-to-use HRP substrate (TMB) is added to the wells. The intensity of the colour produced is directly proportional to the amount of hNRTN in the sample. Colour development is then stopped by the addition of stop solution. Absorbance is measured at 450 nm.

SENSITIVITY

The detection range of the assay is from 50 pg/mL to 1600 pg/mL. The detection limit is 5 pg/mL to 13 pg/mL, defined by the minimum hNRTN 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).

As hNRTN may be unstable in low concentrations (diluted standard stock solution, standards and diluted samples), it is recommended to prepare standards and samples directly before the test procedure.

KIT CONTENTS

- Pre-coated microwell plates: 96 microwells coated with hNRTN-specific mouse polyclonal antibodies
- hNRTN sample diluent, 25 mL, pink solution (citrate buffer, BPLA, detergent and preservative)
- hNRTN standard stock solution, 2 × 30 µl, pink solution (1.6 µg/mL)
- Anti-human NRTN enzyme conjugate stock, 60 µl (HRP-conjugated rabbit polyclonal and mouse monoclonal antibodies in a stabilizing solution)
- Anti-human NRTN enzyme conjugate stock diluent, 12 mL, blue solution (PBS pH7.4, 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 (10−1000 µl)
- (Micro)centrifuge tubes
- Microplate reader (450 nm)
- Lid or sealing tape for microwell plate
- Microwell plate shaker
- Vortex mixer

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	Prepare standards directly before use by serial dilution using standard stock solution and sample diluent (pink). Use vortex mixer to shake the standard stock solution before use!
STEP 3	Perform dilutions of each sample in sample diluent directly before use.
STEP 4	Add 100 µL of samples and standards (pink) into appropriate wells in duplicate.
STEP 5	Incubate the covered microplate for 60 min at RT on a microwell plate shaker (300 rpm).
STEP 6	Prepare enzyme conjugate working solution by diluting enzyme conjugate stock in enzyme conjugate stock diluent (blue) (1:200 – 1:400) (the precise dilution factor comes with the kit).
STEP 7	Discard the solution and wash the wells 4 times with 300 µL of washing solution.
STEP 8	Add 100 µL of enzyme conjugate (blue) into each well.
STEP 9	Incubate the covered microplate for 60 min at RT on a microwell plate shaker (300 rpm).
STEP 10	Discard the solution and wash the wells 4 times with 300 µL of washing solution.
STEP 11	Add 100 µL of substrate solution into each well.
STEP 12	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 13	Stop the reaction by adding 50 µl of STOP solution into each well in the same order and time as for TMB distribution.
STEP 14	Read the absorbance at 450 nm immediately.

Icosagen | Eerika tee 1, Ülenurme vald, Tartumaa 61713, Estonia | www.icosagen.com | Ph: +372 737 7070 | email: info@icosagen.ee

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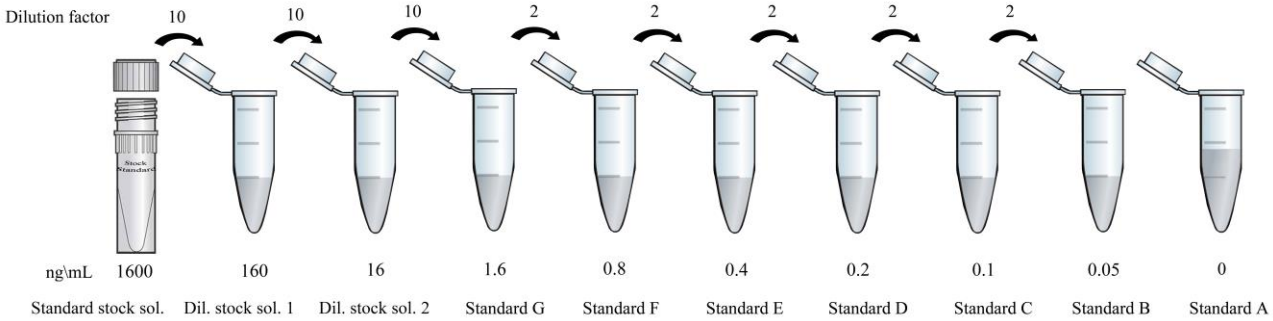
PREPARATION OF STANDARDS



Prepare the standards immediately prior to use by serial dilution according to the table below. Use vortex mixer to shake the standard stock solution before use! Avoid foaming or bubbles when mixing components. The volumes of the standards given in the table are calculated for one assay (standard curve) only. Discard any remaining standards after use.

Standard	Conc. of hNRTN	Vol. of hNRTN solution (µL)	Vol. of sample diluent (µL)
Standard stock solution	1.6 µg\ml		
Diluted stock solution 1	160 ng\ml	10 µl of standard stock solution	90 µl
Diluted stock solution 2	16 ng\ml	10 µl of diluted stock sol. 1	90 µl
G	1.6 ng\ml	50 µl of diluted stock sol. 2	450 µl
F	800 pg\ml	250 µl of standard G	250 µl
E	400 pg\ml	250 µl of standard F	250 µl
D	200 pg\ml	250 µl of standard E	250 µl
C	100 pg\ml	250 µl of standard D	250 µl
B	50 pg\ml	250 µl of standard C	250 µl
A	0 pg\ ml		250 µl

PREPARATION OF SAMPLES

Dilute the samples in sample diluent.

PREPARATION OF ENZYME CONJUGATE WORKING SOLUTION

The enzyme conjugate working solution should be prepared up to 30 min before use. Prepare the enzyme conjugate working solution by diluting enzyme conjugate stock in enzyme conjugate stock diluent (1:200 – 1:400) (the precise dilution factor comes with the kit). Discard any remaining working solution after use.

MICROWELL PLATE WASH

It is recommended that the microwell plate wells be washed by hand (e.g. using a multi-channel pipette) during the washing steps, as a plate washer may cause poor assay precision.

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.

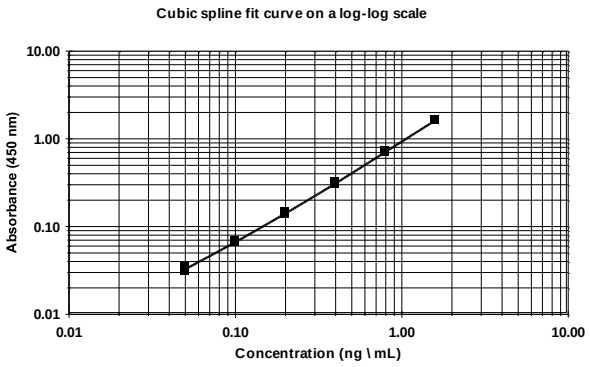
The hNRTN concentration in the sample can be calculated by interpolation between standard points on the curve.

When generating a four parameter logistic fit curve instead of a cubic spline fit curve only minor differences occur in hNRTN 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 1.3 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).



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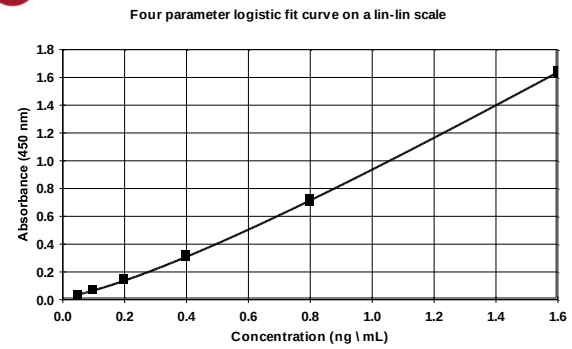

PRECISION

Intra-assay precision:

Sample	Number of measures	Mean (pg/mL)	CV%
1	16	1180	2.0
2	16	2010	2.7
3	16	2770	2.2

Inter-assay precision:

Sample	Number of assays	Mean (pg/mL)	CV%
1	5	580	8.5
2	5	1094	9.8
3	5	2794	6.5

LINEARITY (DILUTION TEST)

Three plasma samples and one cell culture supernatant were diluted with sample diluent. The concentration of hNRTN 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 factor	Conc. (pg\mL)	%
#1	4	680	100
	5	640	94
	6	UD	UD
	8	UD	UD
#2	4	720	100
	5	750	104
	6	690	96
	8	850	118
#3	4	2040	100
	5	2120	104
	6	2110	103
	8	2100	103

UD – Undetectable (The AU of the sample is below the detection range)

Sample	Dilution factor	Conc. (ng\mL)	%
Cell culture supernatant	10 000	14438	100
	20 000	14689	102
	40 000	16045	111
	80 000	16528	115

RECOVERY

hNRTN standards from 100 pg/ml to 3200 pg/mL were added to equal volumes of three plasma samples containing different concentrations of hNRTN. hNRTN standards from 100 pg/ml to 3200 pg/mL were added to equal volume of one cell culture supernatant sample. The theoretical concentration and the recovered concentration were calculated.

Sample	Added conc. (pg/mL)	Expected conc. (pg/mL)	Obtained conc. (pg/mL)	Recovery %
#1	0		130	100
	500	380	440	116
	2000	1130	1140	101
	4000	2130	2090	98
#2	0		200	100
	500	450	470	104
	2000	1200	1150	96
	4000	2200	2020	92
#3	0		420	100
	500	670	650	97
	2000	1420	1270	89
	4000	2420	2200	91

Sample	Added conc. (pg/mL)	Expected conc. (pg/mL)	Obtained conc. (pg/mL)	Recovery %
Cell culture supernatant	0		790	100
	100	840	880	105
	200	890	950	107
	400	990	1050	106
	800	1190	1270	107
	1600	1590	1750	110
	3200	2390	2580	108

TECHNICAL ASSISTANCE

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