HUMAN CNTF ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CNTF CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:

ELISA NAME	**HUMAN CNTF ELISA**
Catalog No.	SK00751-01
Lot No.	
Formulation	96 T
Standard range	46.8 - 3000 pg/mL
Sensitivity	23.4 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factors	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human CNTF
Calibration	Human CNTF recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Human CNTF ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CNTF from serum and plasma (other samples need to be validated prior to assay) in a sandwich ELISA format.

This immunoassay contains recombinant human CNTF and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CNTF samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human CNTF. The capture antibody can bind to the human CNTF in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human CNTF is added to the wells. After another washing of the plate, UltraAvidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human CNTF bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
CNTF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human CNTF.	**751-01-01**	**1 plate**
CNTF Standard – 3000 pg/vial of recombinant human CNTF in a buffered protein base with preservative; lyophilized.	**751-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against CNTF with preservative; lyophilized.	**751-01-03**	**1 vial**
Positive Control – one vial of recombinant human CNTF; lyophilized.	**751-01-04**	**1 vial**
UltraAvidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of UltraAvidin conjugate to HRP.	**UAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody & HRP Diluent Solution – 30 mL of buffered protein based solution with preservative.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody

concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. UltraAvidin-HRP Conjugate 200-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

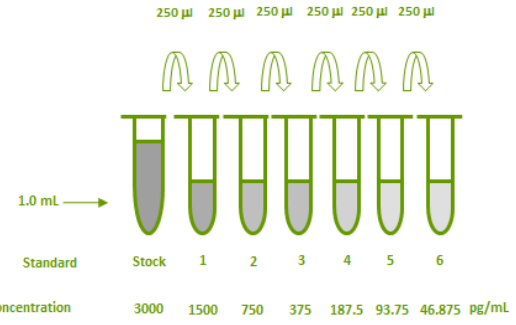
Serum or EDTA samples DO NOT require dilution. If sample values are higher than the maximum standard then a 2 or 4-fold dilution or greater dilution is needed. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. A suggested 4-fold dilution is 60 µL sample + 180 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

CNTF Standard - Reconstitute the CNTF standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **3000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	3000 pg/ml
# 1	250 µl of stock	250 µl	1500 pg/ml
# 2	250 µl of 1	250 µl	750 pg/ml
# 3	250 µl of 2	250 µl	375 pg/ml
# 4	250 µl of 3	250 µl	187.5 pg/ml
# 5	250 µl of 4	250 µl	93.75 pg/ml
# 6	250 µl of 5	250 µl	46.875 pg/ml



Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C or -70° C.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody & HRP Diluent Solution (DB08)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody & HRP Diluent Solution into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

UltraAvidin-HRP Conjugate - Pipette 11.94 mL of **Antibody & HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of UltraAvidin-HRP Conjugate should be used within a few days. **Protect from light.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions in reverse order of serial dilution, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of UltraAvidin-HRP Conjugate working solution to each well. Incubate it on microplate shaker for 45 minutes at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-7 minutes on microplate shaker at room temperature. **Protect from light. *Be prepared to add Stop Solution quickly due to quick color development.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CNTF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 3000 pg/mL may result in inaccurate, low human CNTF levels. Such samples require further external predilution according to expected human CNTF values with Dilution Buffer in order to precisely quantify the actual human CNTF level.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.102)
46.875	0.068
93.75	0.129
187.5	0.277
375	0.485
750	0.922
1500	1.402
3000	1.853

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human CNTF	100
Human BDNF	< 5
Human β-NGF	< 5
Human GDNF	< 5
Mouse β-NGF	< 5
Mouse GDNF	< 5
Rat GDNF	< 5

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl UltraAvidin-HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate 3-7 min on the plate shaker at RT. **Protect from light.**

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Add 100 µl Stop Solution to each well. Read 450nm within 15 min.