HUMAN BETA NERVE GROWTH FACTOR (β-NGF) ELISA KIT

PURCHASE INFORMATION:

FOR THE QUANTITATIVE DETERMINATION OF HUMAN β-NGF CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, EDTA PLASMA

ELISA NAME	HUMAN β-NGF ELISA
Catalog No.	SK00307-01
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µl
Sample Type	Cell Culture Supernates, Serum, EDTA Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human β-NGF
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2°C - 8°C

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INTRODUCTION

Human β-NGF immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human β-NGF in cell culture supernates, serum, and EDTA plasma. It contains recombinant human β-NGF and antibodies raised against this protein. It has been shown to accurately quantify recombinant human β-NGF. Results obtained with naturally occurring β-NGF samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human β-NGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for β-NGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any β-NGF present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for β-NGF is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of β-NGF bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_The kit should not be used beyond the expiration date on the kit label.

_Do not mix or substitute 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.

_If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
β-NGF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against β-NGF.	307-01-01	1 plate
β-NGF Standard – 1000 pg/vial of recombinant human β-NGF in a buffered protein base with preservatives; lyophilized.	307-01-02	1 vial
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrate of biotinylated polyclonal antibody against β-NGF with preservatives; lyophilized.	307-01-03	1 vial
Positive Control - one vial of recombinant human β-NGF, lyophilized	307-01-04	1 vial
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	DB01	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 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted standard and detection antibody concentrate solution could be stored for up to a month at -70°C.

Diluted standard working solution and positive control should be prepared and used immediately. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. 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.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.
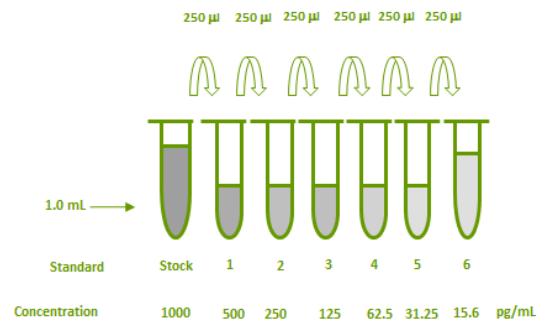
REAGENT PREPARATION

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 Wash Buffer.

β-NGF Standard - Refer to vial label for reconstitution volume. Reconstitute the β-NGF standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 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 1000 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	1000 pg/ml
# 1	250 µl of stock	250 µl	500 pg/ml
# 2	250 µl of 1	250 µl	250 pg/ml
# 3	250 µl of 2	250 µl	125 pg/ml
# 4	250 µl of 3	250 µl	62.5 pg/ml
# 5	250 µl of 4	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.625 pg/ml

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer 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 Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE
Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 μL of **Dilution Buffer** to Blank wells (E4, E5).
4. Add 100 μL of **Standard** (from B2, B3 to G2, G3 and G4, G5), **sample, or positive control** (F4, F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
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 micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker 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 8-12 minutes at room temperature. **Protect from light.**
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 micro-plate reader set to 450 nm.
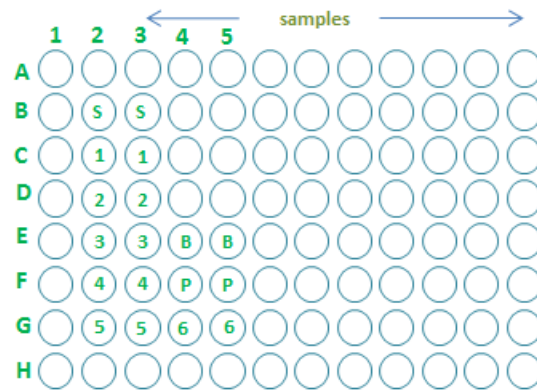
CALCULATION OF RESULTS
Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log- log curve fit. As an alternative, construct a 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 β-NGF 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.

CALIBRATION
This immunoassay is calibrated against a highly purified NS0-expressed recombinant human β-NGF.

SENSITIVITY
Twenty-five assays were evaluated and the minimum detectable dose (MDD) of β-NGF was 5 pg/mL.

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

β-NGF Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.079)
7.813 (optional)	0.014
15.625	0.024
31.25	0.038
62.5	0.071
125	0.138
250	0.270
500	0.546
1000	1.052

- Lot No.:
- Positive Control: 50 – 150 pg/mL

SPECIFICITY

The factors listed below were prepared at 50 ng/mL in Dilution Buffer and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY (%)
Human β-NGF	100
Mouse β-NGF	32.4
Rat β-NGF	2.7
Human BDNF	0
Human CNTF	0
Human GDNF	0
Human NT-3	0
Rat CNTF	0
Rat GDNF	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

⬇

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

⬇

Aspirate and wash 4 times.

⬇

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

⬇

Aspirate and wash 4 times.

⬇

Add 100 μL Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**

⬇

Aspirate and wash 4 times.

⬇

Add 100 μL Substrate Solution to each well. Incubate 8-12 minutes on the plate shaker. **Protect from light**.

⬇

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