BioAim Scientific Inc

Human Beta-NGF EasyTest™ ELISA Kit

Cat.No: 1010005

Instruction Manual

For research use only

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I. INTRODUCTION

NGF is composed of three non-covalently linked subunits, α, β, and γ. Both the α and γ subunits of NGF are members of the kallikrein family of serine proteases while the β subunit, called β-NGF exhibits all the biological activities ascribed to NGF. Recombinant human β-NGF is a homodimer of two 120 amino acid polypeptides. The human protein shares approximately 90% homology at the amino acid level with both the mouse and rat β-NGF and exhibits cross-species activity.

NGF is critical for the survival and maintenance of sympathetic and sensory neurons. Without it, these neurons undergo apoptosis. Nerve growth factor causes axonal growth. NGF binds with at least two classes of receptors: p75NTR and TrkA. TrkA dimerizes and autophosphorylates its tyrosine kinase segment, which leads to the activation of PI 3-kinase, ras, and PLC signaling pathways. Alternatively, the p75NTR receptor can form a heterodimer with TrkA which has higher affinity and specificity for NGF. In addition, NGF can also act in the central nervous system as a neurotrophic factor for basal forebrain cholinergic neurons. NGF has also been shown to have biological effects on non-neuronal tissues. NGF has been found to increase the number of mast cells in neonatal rats and to induce histamine release from peritoneal mast cells. NGF will also induce the growth and differentiation of human B lymphocytes as well as suppress apoptosis of murine peritoneal neutrophils. These results suggest that NGF may have an important role in the regulation of the immune system.

The BioAim Human Beta-NGF EasyTest™ ELISA kit can quantitatively measure Beta-NGF in human serum or plasma. It is a simple and rapid technology for the quantitation of antigen in a range of sample matrices. The whole process takes less than 1.5 hours with high accuracy and precision. EasyTest™ ELISA is faster and easier to perform than standard format ELISA with less reagent handling and fewer pipetting steps.

II. REAGENTS

1. Human Beta-NGF Microplate: 96 breakable wells (12strips x 8wells) coated with anti-human Beta-NGF.
2. 20x Wash Buffer Concentrate: 1 Vial, 25 ml.
3. 5x Assay Diluent: 1vial, 15 ml.
4. Standards: 10µl/ vial, 2 vials, recombinant human Beta-NGF.
5. BioAim human Beta-NGF Mix: 8µl/vial, 4 vials.
6. TBM Substrate solution: 1 Vial, 12 ml.
7. Stop Solution: 1 Vial, 8 ml of 0.2 M sulfuric acid.

III. STORAGE

1. The kit can be stored for up to 6 months at 2º to 8ºC from the date of shipment.
2. Standard can be stored at -20 ºC or -80 ºC. Use freshly prepared standard within 12hours (stored at 2~8 ºC).
3. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 ºC. Return unused strip to the pouch containing desiccant pack, reseal along entire edge and keep in 2~8 ºC.
4. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1. Distilled or deionized water.
2. Precision pipettes, with disposable plastic tips.
3. Beakers, flasks, cylinders necessary for preparation of reagents.
4. Microplate washing device (multichannel pipette or automated microplate washer).
5. Microplate shaker.
6. Microplate reader capable of reading at 450 nm.

V. PRECAUTIONS

1. All reagents must be at room temperature (18 ºC to 25 ºC) before running assay.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Use disposable pipette tips for each transfer to avoid microbial contamination or cross contamination of reagents.
6. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
7. Avoid contact of stop solution with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
8. Do not use TMB substrate solution if it has begun to turn blue.
9. Do not expose bleach to work area during actual test procedure because of potential interference with enzyme activity.

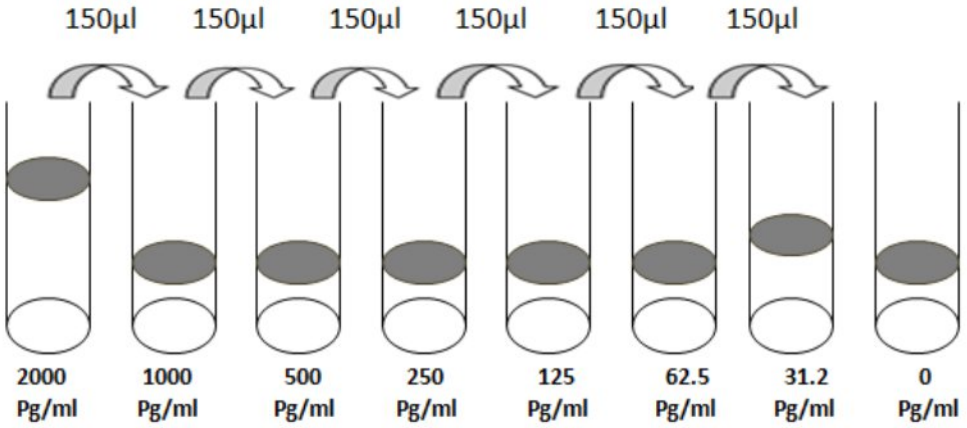
VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18~25°C) before use.
2. **Assay diluent**
   Dilute the concentrated assay diluent 1:5 with distilled water (e.g. 10ml plus 40ml).
3. **Wash Buffer**
   Dilute the concentrated wash buffer 1:20 with distilled water (e.g. 20ml plus 380ml).
4. **Sample**
   Levels of the target protein may vary among different specimens. Optimal dilution factors for each sample must be determined by the investigator.

The dilution scheme is only suggestion: the recommended dilution for serum and plasma is 1: 2.

5. Standard

a. Briefly spin standard vial before use. Add 490 µl 1x Assay Diluent to prepare a 5ng/ml standard. Gently vortex to mix.
b. Take 200 µl Beta-NGF standard into a tube; then add 300 µl 1x Assay Diluent to prepare a 2000 pg/ml stock standard solution.
c. Add 150 µl 1x Assay Diluent to 7 tubes. Label as 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml and the last tube with 1x assay diluent is the blank as 0pg/ml.
d. Perform serial dilutions by adding 150 µl of each standard to the next tube and vortexing between each transfer (see figure below).



6. BioAim human Beta-NGF Mix

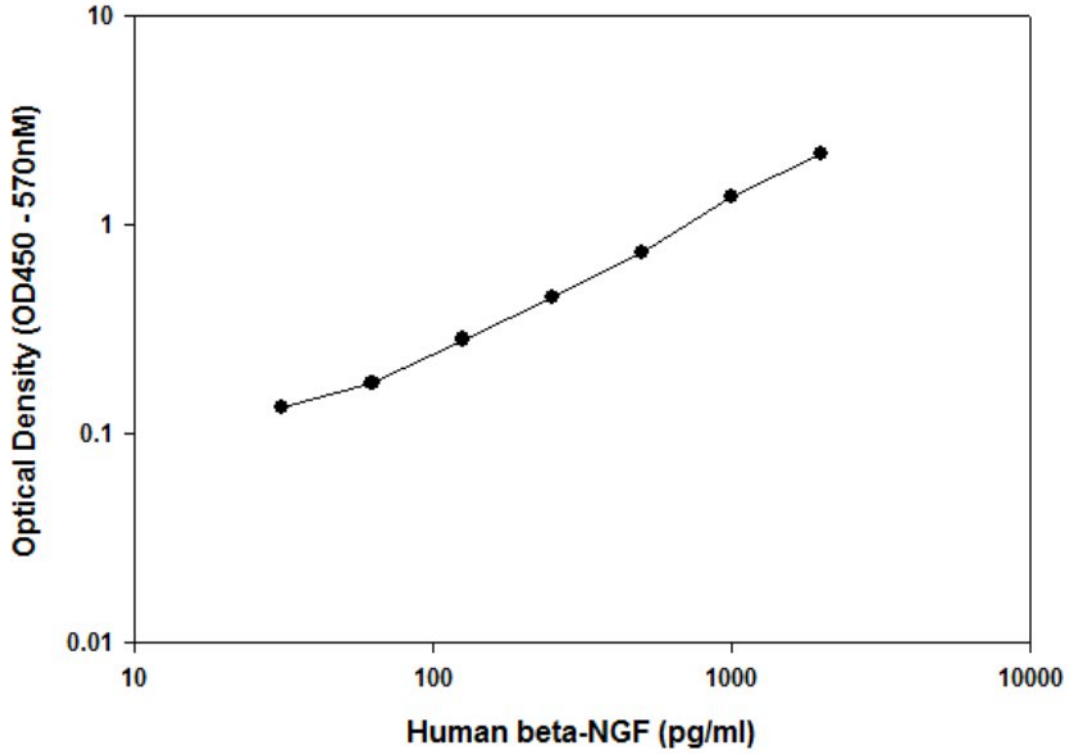
Within15minutes prior to use, briefly spin the vial. Add 1492 µl of 1x Assay diluent to the vial and mix by pipetting. A vial mix can be used for around 30 wells.

VII. ASSAY PROCEDURE

1. All reagents must be brought to room temperature (18-25°C) prior to use. Place the required number of microwells in the holder. It is recommended that all samples, standards, and blanks be run in duplicate.
2. Add 50 µl of 1x Assay Diluent into the blank wells.
3. Add 50 µl of each standard (*see reagent preparation step 5*) and samples into the designated wells. Gently shake/tap the plate for 5 seconds to mix.
4. Add 50 µl of BioAim Beta-NGF Mix into all wells, including the blank wells.
5. Cover wells with plate sealer and incubate at room temperature (18~25°C) for 1 hour with gentle shaking.
6. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µl/well prepared wash buffer followed by decanting/aspirating. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove residual buffer. Thorough washing at this step is very important, complete removal of liquid is required for proper performance.
7. Pipette 100 µl of TMB Substrate Solution to each well. Incubate plate for 15 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of stop solution to each well.
9. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.

VIII. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards, samples and controls. Subtract the average zero standard optical density.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the Beta-NGF concentration on log-log graph paper or using Sigma plot software. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating Beta-NGF for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Beta-NGF concentration.
4. A representative standard curve is shown below. This standard curve is for demonstration only. A standard curve must be run with each assay by operator.



IX. PERFORMANCE

A. Sensitivity

The minimum detectable dose of Beta-NGF was determined to be less than 1pg/ml. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standards.

B. Recovery

Recovery was determined by spiking various levels of Human Beta-NGF into the diluted sample types listed below. Mean recoveries are as follows:

Sample Type	Average % recovery	Range %
Serum	95	89-107
Plasma	103	99-107

C. Linearity

Sample	Dilution	% of expected
Seum	1:2	91
	1:4	83
	1:8	71
Plasma	1:2	94
	1:4	85
	1:8	89

D. Specificity

No cross-reactivity was identified with the following cytokines: Adiponectin, Angiopoietin-1, BDNF, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin, MCP-1, PDGF, RANTES, SCF, TGF-beta, TIMP-2, TNF-alpha, TNF-beta, and VEGF.

E. Reproducibility
Intra-Assay CV%: <10%
Inter-Assay CV%: <15%

X. REFERENCES

1. Fiore M, etal. (2009). Rev Neurosci **20** (2): 133–45.
2. Madduri S, etal. (September 2009). Neurosci. Res. **65** (1): 88–97.
3. Kaplan DR, etal. Nature **350**(6314): 158–60.
4. Crowder RJ, etal. (April 1998). J. Neurosci. **18** (8): 2933–43.
5. Tuszynski MH,etal. (2004). Prog. Brain Res. **146**: 441–9.
6. Villoslada P,etal. (2004). Prog. Brain Res. **146**: 403–14.
7. Chaldakov GN, etal. Riv Psichiatr **44** (2): 79–87.

XI. Troubleshooting

Problem	Cause	Solution
1.Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. check pipettes; 2. Ensure briefly spin the vial of standard, take the right amount to dilution.
2. Low signal	1. Too brief incubation time 2. Inadequate reagent volumes or improper dilution	1. ensure adequate incubation time; 2. Check pipettes and ensure corrected preparation.
3. Large CV	Inaccurate pipetting	1. Check pipettes; 2. Accurately perform each step.
4.High background	1. Plate is insufficiently washed; 2. Wash buffer contamination	1.Follow the manual correctly; if using a plate washer, check that all ports are working functionally; 2. Prepare fresh buffer.
5. Low sensitivity	1.ELISA kit improper storage 2. Stop solution	1. Follow the manual to store each component correctly; 2. Add enough stop solution to each well.

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