Human NGF/NGFβ
ELISA

Catalog # LF-EK50285 (1 kit)

Catalog # LF-EK50286 (4 kits bundle)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of human NGF/NGFβ

For research use only
Not for diagnostic or therapeutic purposes

Contents

1. Introduction

Nerve growth factor (NGF) is a polypeptide involved in the regulation of growth and differentiation of sympathetic and certain sensory neurons. NGF is thought to have a profound effect on the development and maintenance of sympathetic and embryonic sensory neurones. NGF activity isolated from the male mouse submaxillary gland (MSG) consists of three types of subunits, alpha, beta and gamma, which specifically interact to form a 7S, approximately 130,000-molecular weight (Mr) complex. The 7S complex contains two identical 118-amino acid beta-chains, which are solely responsible for the nerve growth-stimulating activity of NGF.[1] NGF, which is expressed by inflammatory cells and effects changes that lead to increased neural responsiveness, could be a pivotal mediator in allergic rhinitis.[2] The standard product used in this kit is human 2.5S NGF, which is a dimmer linking with two polypeptide chains of 120 amino acids.

2. Principles of Method

AbFrontier's human NGF ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human NGF specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human NGF amount of sample captured in plate..

3. Intended Use

The AbFrontier human NGF ELISA kit is to be used for the in vitro quantitative determination of human NGF in sera, plasma, body fluids, tissue lysates or cell culture supernates.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

4. Storage and Stability

All kit components of this kit are stable at 2 to 8℃. Any unused reconstituted standard should be discarded or frozen at -20℃. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.

6. Kit Contents

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Standard Protein	2	10ng/tube
Secondary Antibody	1	130 ul
Avidin-Biotin-Peroxidase Complex (ABC)	1	130 ul
Sample diluent Buffer	1	30 ml
Antibody diluent buffer	1	12 ml
ABC diluent buffer	1	12 ml
TMB color developing agent	1	10 ml
TMB stop solution	1	10 ml

① 96 Well Plate

: Human NGF microtiter plate, one plate of 96 wells.

A plate using break-apart strips coated with a monoclonal antibody specific to human NGF.

② Standard Protein

: Lyophilized human NGF.

③ Secondary Antibody

: Biotin labeled anti human NGF antibody.

④ AV-HRP

: Avidin-Biotin-Peroxidase Complex (ABC)

⑤ Substrate (Stabilized chromogen)

: Tetramethylbenzidine (TMB) solution

⑥ Stop Solution

: 1 N solution of sulfuric acid (H_2SO_4)

Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃ for 30 min before using.

7. Materials Required But Not Provided

① Microtiter plate reader in standard size.
② Automated plate washer.
③ Distilled or deionized water
④ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
⑤ Data analysis and graphing software
⑥ Vortex mixer
⑦ Polypropylene tubes for diluting and aliquoting standard
⑧ Absorbent paper towels
⑨ Calibrated beakers and graduated cylinders of various sizes
⑩ Washing buffer (neutral PBS or TBS). Preparation of 0.01M **TBS:** Add 1.2g Tris, 8.5g Nacl; 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS:** Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

8. Reagent Preparation

1) Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

- **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 2000 X g for 20 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma**: Collect plasma using heparin as an anticoagulant. Centrifuge for 10 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

2) Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the

middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

- o **High target protein concentration (10-100ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- o **Medium target protein concentration (1-10ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- o **Low target protein concentration (15.6-1000pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- o **Very Low target protein concentration (≤15.6pg/ml).** No dilution necessary, or the working dilution is 1:2.

3) Reagent Preparation and Storage

A. Reconstitution of the human NGF standard： NGF standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of NGF standard (10ng per tube) are included in each kit. Use one tube for each experiment.

a. 10,000pg/ml of human NGF standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 1000pg/ml of human NGF standard solution: add 0.1m of the above 10ng/ml NGF standard solution into 0.9ml sample diluent buffer and mix thoroughly.

c. 500pg/ml→15.6pg/ml of human NGF standard solutions: Label 6 Eppendorf tubes with 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 1000pg/ml NGF standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human NGF antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Biotinylated anti-human NGF antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

9. Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard NGF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of NGF amount in samples.

1. Aliquot 0.1ml per well of the 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml human NGF standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human serum, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human NGF standard solution and each sample is measured in duplicate.

2. Seal the plate with the cover and incubate at 37°C for 90 min.

3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. Add 0.1ml of biotinylated anti-human NGF antibody working solution into each well and incubate the plate at 37°C for 60 min.

5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method**: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)

6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer

stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).

8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 20-25min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human NGF standard solutions; the other wells show no obvious color).

9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.

10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{.450}$) = (the $O.D._{.450}$ of each well) – (the $O.D._{.450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{.450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human NGF concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in for 20-25 min.
5. Add TMB stop solution and read.

10. Characteristics

1) Typical result

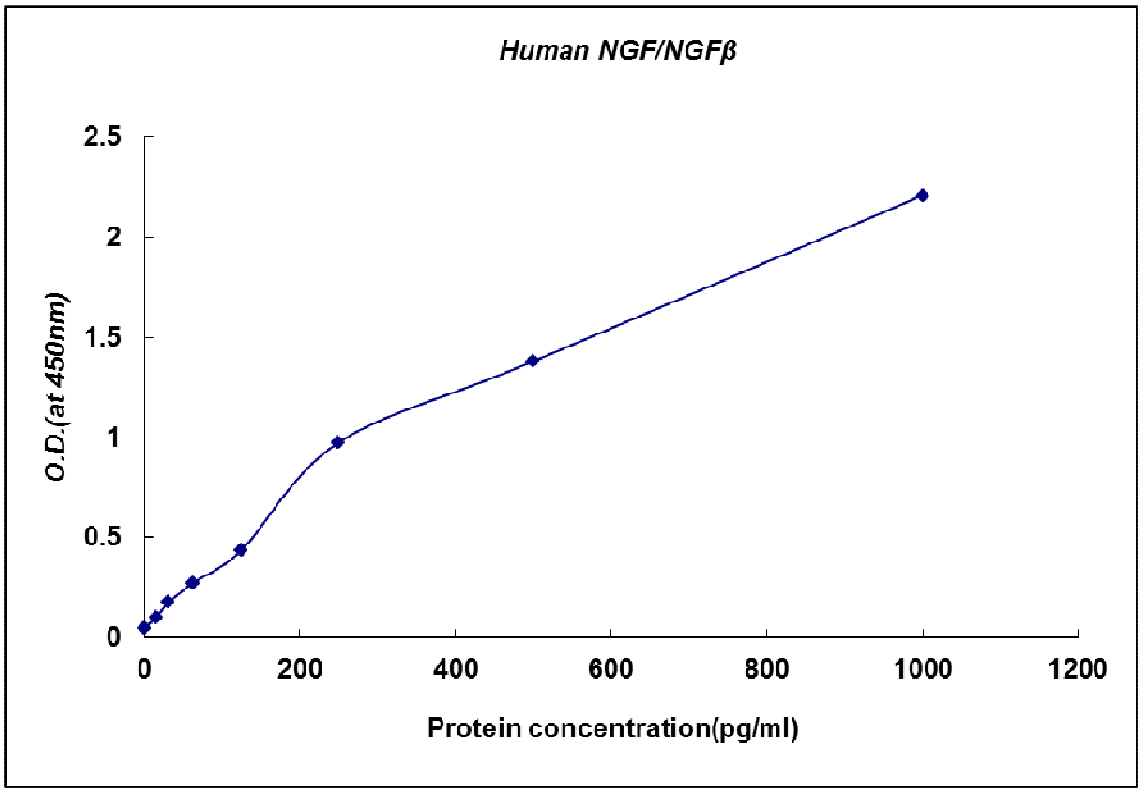
Typical Data Obtained from Human NGF

(TMB reaction incubate at 37℃ for 20 min)

Standard Human NGF (pg/ml)	Optical Density (at 450nm)
0	0.049
15.6	0.101
31.3	0.174
62.5	0.272
125	0.436
250	0.975
500	1.381
1000	2.210

Typical Human NGF ELISA kit Standard Curve

This standard curve was generated at AbFrontier for demonstration purpose only. A standard curve must be run with each assay.



2) Sensitivity: < 1pg/ml

3) Specificity: No detectable cross-reactivity with any other cytokine.

11. Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much AV-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30 ℃ before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing – unbound AV-HRP remaining	• Increase number of washes carefully
	• Too much AV-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

12.Reference

1) Ullrich, A.; Gray, A.; Berman, C.; Dull, T. J. Human beta-nerve growth factor gene sequence highly homologous to that of mouse. Nature 303: 821-825, 1983.

2) Sanico, A. M.; Stanisz, A. M.; Gleeson, T. D.; Bora, S.; Proud, D.; Bienenstock, J.; Koliatsos, V. E.; Togias, A. Nerve growth factor expression and release in allergic inflammatory disease of the upper airways. Am. J. Resp. Crit. Care Med. 161: 1631-1635, 2000.

◈ Ordering Information

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