Rat NGF/NGFBETA ELISA Kit
Cat. No. CL0471
96-wells

Target Protein Species: Rat
Range 15.6 pg/ml – 1000pg/ml
Specificity No detectable cross-reactivity with BDNF, GDNF, CNTF, NT3, and NT4.

**CELL APPLICATIONS, INC.**

KIT COMPONENTS

Component	Amount
96-well plate precoated with anti-Rat NGF antibody	1 Plate
Protein Standard: Lyophilized recombinant Rat NGF	2 tubes, 10 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-Rat NGF)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethyllbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

Storage
Store at 4℃. Cell Applications, Inc. recommends using the kit within 6 months of order.
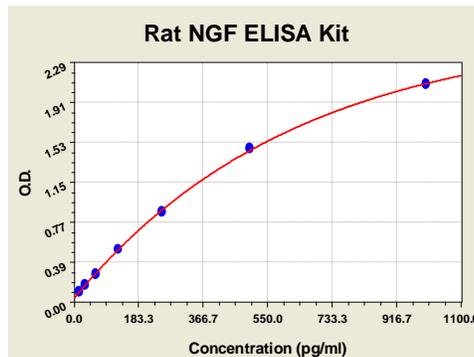
Rat NGF ELISA Kit

X	pg/ml	0.0	15.6	31.3	62.5	125	250	500	1000
Y	O.D.450	0.045	0.108	0.173	0.282	0.507	0.868	1.472	2.084

Figure 1: NGF **Standard Curve.** Using the Rat NGF ELISA Kit, O.D. data was graphed against NGF protein concentration. The TMB reaction was incubated at 37° C for 22 min.

BACKGROUND

Nerve Growth Factor (NGF) is a small secreted protein and a member of neurotrophin family, which also includes Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), and Neurotrophin 4/5 (NT-4/5). All the neurotrophins are initially produced as 30–35 kDa precursor proteins containing a signal peptide, sites for glycosylation, and pairs of basic amino acids that are recognized by processing enzymes. The calcium-dependent serine protease furin and other members of the prohormone convertase family cleave each of the neurotrophins at a dibasic cleavage site in the middle of the precursor protein, releasing the biologically active 12–14 kDa C-terminal product. NGF plays important roles in the growth, maintenance, and survival of certain target sympathetic and sensory neurons. Without it, these neurons undergo apoptosis. NGF causes axonal growth. Studies have shown that it causes axonal branching and a bit of elongation. NGF plays a role in the repair, regeneration, and protection of neurons. Thus, it could serve as a therapeutic agent in neurodegenerative conditions such as Alzheimer's disease. NGF has also been suggested to play a role in other physiological systems and tissues such as the immune system. There is evidence that NGF circulates throughout the entire body and is important for maintaining homeostasis. There is also evidence that shows that the precursor to NGF, pro-NGF, may also play important roles due to its abundance. These include apoptotic and neurotrophic properties.[1] NGF binds with at least two classes of receptors: p75NTR (low affinity nerve growth factor receptor, which belong to the death receptor family) and TrkA, a transmembrane tyrosine kinase. NGF may signal its neuroprotective actions through the tyrosine kinase TrkA receptor and trigger apoptosis in some cells through the p75 receptor in absence of TrkA. High-affinity binding of NGF requires both TrkA and p75NTR.[2] The downstream effectors of the ras pathway include activation of fos and jun to form AP-1, activating genes through this transcription factor. Other transcription factors involved in NGF responses include Egr and CREB. The Egr family of transcription factors as well as the Mek/Erk pathway contribute to NGF-induced neurite formation. The CREB family of transcription factors are involved in NGF-induced survival of sympathetic neurons.[3]

Reference

1. Sofroniew, M.V. et al: Ann. Rev. Neurosci. 24:1217-81, 2001
2. Delcroix, J-D. et al: Neuron 39:69-84, 2003
3. Barbacid, M.: J. Neurobiol. 25:1386-403, 1994

ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethyllbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

NOTES:

• Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
• Duplicate assay wells are recommended for both standard and sample testing.
• Do not let the 96-well plate dry, this will lead to inactivation of plate components.
• When diluting samples and reagents, ensure that they are mixed completely and evenly.
• Pre-warm diluted ABC and TMB solutions at 37℃ for 30 min before use to avoid variable temperature effects.
• For washes, use TBS or PBS. Do not touch well walls.
• A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
• The user will determine sample dilution fold by estimation of target protein amount in samples.

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ELISA PROTOCOL

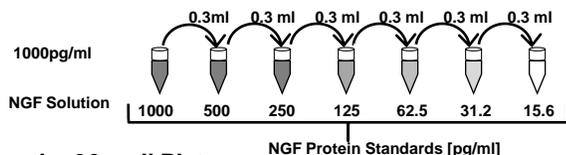### Preparation of Test Samples

1. Process Test Samples in the following manner:
   - **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 30 min) at room temperature. Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20° C..

2. Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the 15.6-1000 pg/ml standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

Target Protein Concentration Range	Sample Working Dilution	Sample Vol.	Diluent Buffer Vol.
10-100 ng/ml	1:100	1 µl	99 µl
1-10 ng/ml	1:10	10 µl	90 µl
15.6-1000 pg/ml	1:2	50 µl	50 µl
≤15.6pg/ml	n/a	100µl	n/a

3. If samples will be assayed within 24 hours, store at 2-8° C. For long-term storage, aliquot and freeze samples at -20° C. Avoid repeated freeze-thaw cycles.

Preparation of Standard Solutions (15.6-1000 pg/ml)

4. Reconstitute the Lyophilized Recombinant Protein to make a 10.000 pg/ml Rat NGF solution. Add 1 ml Sample Diluent Buffer to a tube of lyophilized protein, keep the tube at room temperature for 10 min. Mix thoroughly.

5. Add 0.1 ml of the mixed 10,000 pg/ml NGF solution to the eppendorf tube containing 0.9 ml diluent buffer and mix to make a 1000 pg/ml NGF solution.

6. Label 6 eppendorf tubes with the Rat NGF protein concentrations to be prepared by serial dilution :500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml

7. Aliquot 0.3 ml of the Sample Diluent Buffer to the labeled tubes.

8. Serially dilute the protein standards into their respectively labeled tubes. Transfer 0.3 ml from the 1000pg/ml NGF Solution to the 500pg/ml eppendorf tube and mix thoroughly. Transfer 0.3 ml of the 500 pg/ml solution to the 250pg/ml tube and mix thoroughly, Transfer 0.3 ml of the 250pg/ml solution to the 125pg/ml tube and mix, and so on to make the 62.5, 31.3 and 15.6 pg/ml solutions.

9. Store at 4° C until use.

NGF Protein Standards [pg/ml]

Loading the 96-well Plate

10. Aliquot 0.1 ml of the sample diluent buffer into a control well to serve as the Blank. This will yield the O.D.450(Blank) reading.

11. Aliquot 0.1 ml of the standard solutions of the **Preparation of Standard Solutions** (15.6-1000pg/ml) into empty wells of the precoated 96-well plate. Duplicate measurements of standards are recommended.

12. Aliquot 0.1 ml of each properly diluted test sample to empty wells prepared in **Step 2**. Duplicate measurements of each test sample are recommended.

13. Cover the 96-well plate and incubate at 37° C for 90 min.

14. During the **Step 13** incubation period, prepare a stock of Biotinylated 1:100 Antibody Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the Biotinylated Antibody to 1:100 in Antibody Diluent Buffer and mix thoroughly. Use the working solution within 2 hours.

15. Upon completion of the 90 min incubation of **Step 13**, remove the cover of the 96 well plate and discard plate well contents. Blot the plate onto paper towels or other absorbent material. DO NOT let the wells completely dry at any time.

16. Add 0.1 ml of the Biotinylated 1:100 Antibody Working Solution (prepared in **Step 14**) to each well and incubate the plate at 37° C for 60 min.

17. During the incubation period of **Step 16**, prepare a stock of ABC Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the ABC Stock Solution to 1:100 in ABC Diluent Buffer and mix thoroughly. Pre-warm the ABC working solution at 37° C for 30 min before use. Use the working solution within 1 hour.

18. Upon completion of the 60 min incubation of **Step 16**, wash the plate 3 times with 0.3 ml TBS or PBS. For each wash, leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

19. Add 0.1 ml of prepared ABC Working Solution (prepared in **Step 17**) to each well and incubate the plate at 37° C for 30 min.

20. During the incubation period of **Step 19**, pre-warm TMB Color Developing Agent at 37° C for 30 min before use.

21. Upon completion of the 30 min incubation of **Step 19**, wash the plate 5 times with 0.3 ml TBS or PBS. For each wash, leave the washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

22. Add 90 µl of the pre-warmed TMB Color Developing Agent into each well and incubate at 37° C for 20-25 min (shades of blue can be seen in the wells with the four most concentrated Protein Standard Solutions; the other control wells should show no obvious color).

23. Add 0.1 ml of the TMB Stop Solution to each well. The acidic stop solution will change the mixture color to yellow. The yellow intensity is proportional to the amount of target protein captured by the plate.

24. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution. These readings are the O.D.450(Reading).

Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)

- Plot the standard curve: plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.

- The Rat NGF concentration of the samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.

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