

APA105Hu01 100µg
Active Nerve Growth Factor (NGF)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Glu19~Arg239

Tags: N-terminal His-tag

Purity: >98%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% sarcosyl, 5% trehalose.

Applications: Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 10.1

Predicted Molecular Mass: 29.2kDa

Accurate Molecular Mass: 30&33kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

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EP HSESNVPAGH TIPQAHWTKL QHSLDTALRR  
ARSAPAAAIA ARVAGQTRNI TVDPRLFKKR RLRSPRVLFS TQPPREAADT  
QDLDFEVGGA APFNRTHRSK RSSSHPIFHR GEFSVCDSVS VWVGDKTTAT  
DIKGKEVMVL GEVNINNSVF KQYFFETKCR DPNPVDGCR GIDSKHWNSY  
CTTHTFVKA LTMDGKQAAW RFIRIDTACV CVLSRKAVR
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[ACTIVITY]

Mechanism: Nerve growth factor (NGF) is a neurotrophic factor and neuropeptide primarily involved in the regulation of growth, maintenance, proliferation, and survival of certain target neurons. When the pheochromocytoma cell line PC12 is exposed to nerve growth factor (NGF), the cells respond over a period of a week by ceasing cell division and extending neurites (Greene and Tischler, 1976). The cells were grown in Ham's F12K containing 5% fetal calf serum and 10% horse serum on polystyrene tissue culture plates or collagen coated plates. When cells reached log phase growth and fresh medium was added together with 10ng/mL of NGF, then cells were observed by inverted microscope everyday.

Result 1: The cell division ceased and morphological differentiation of PC12 cells was observed obviously after incubation with NGF (10ng/mL) for 6 days. Control group which received no NGF displayed no neurite outgrowth and cells multiply rapidly.

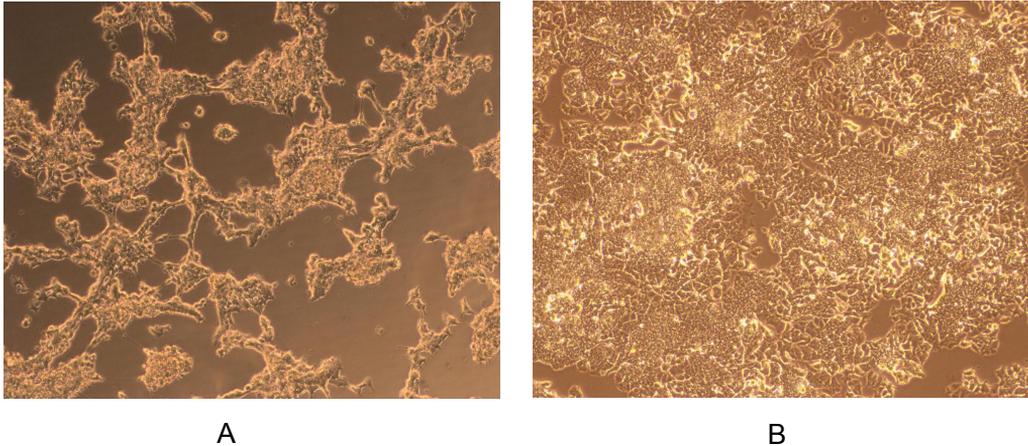


Figure 1. Effect of NGF on PC12 cells.

(A) PC12 cells cultured in Ham's F12K containing 5% fetal calf serum and 10% horse serum on collagen coated plates, stimulated with 10ng/mL NGF for 6 days;

(B) Unstimulated PC12 cells cultured in Ham's F12K containing 5% fetal calf serum and 10% horse serum on collagen coated plates.

NGF is also involved in pathways besides those regulating the life cycle of neurons. Besides, Alpha-2-Macroglobulin (a2M) has been identified as an interactor of NGF, thus a binding ELISA assay was conducted to detect the interaction of recombinant human NGF and recombinant human a2M. Briefly, NGF were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ L were then transferred to a2M-coated microtiter wells and incubated for 2h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-NGF pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^{\circ}$ C. Finally, add 50 μ L stop solution to the wells and read at 450nm immediately. The binding activity of NGF and a2M was shown in Figure 2, and this effect was in a dose dependent manner.

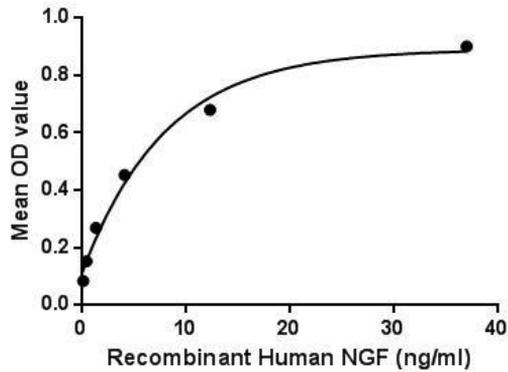


Figure 2. The binding activity of NGF with a2M

[IDENTIFICATION]

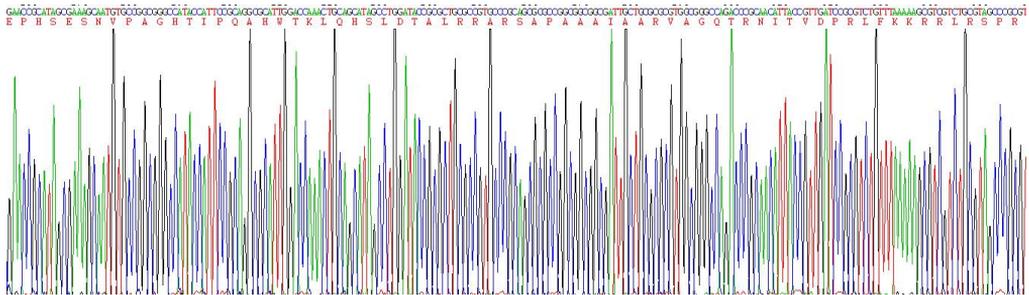


Figure 3. Gene Sequencing (extract)

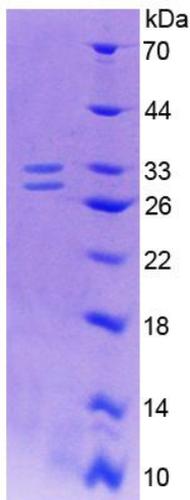


Figure 4. SDS-PAGE

Sample: Active recombinant NGF, Human

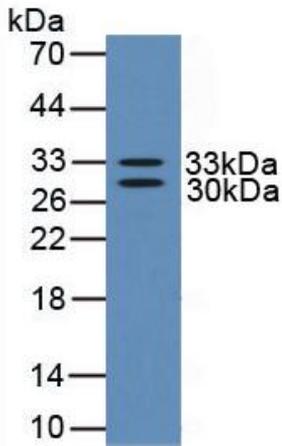


Figure 5. Western Blot

Sample: Recombinant NGF, Human;

Antibody: Rabbit Anti-Human NGF Ab (PAA105Hu01)

[**IMPORTANT NOTE**]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.