APA105Hu01 50μg
Active Nerve Growth Factor (NGF)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Glu19~Arg121+DDDDK+Ser122~Arg239
Tags: N-terminal His-tag
Purity: >98%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5% Trehalose.
Original Concentration: 400μg/mL
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: 29.0kDa
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 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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.

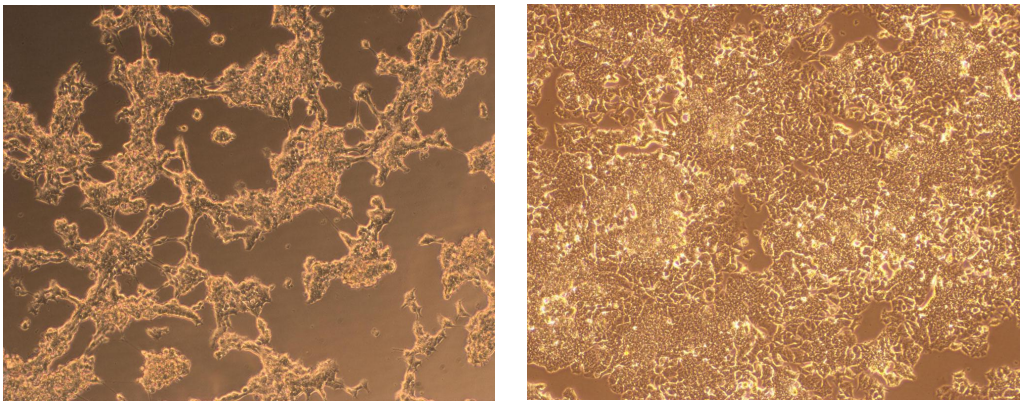
[SEQUENCE]

EPHSESNVPAGHTIPQAHWTKLQHSLDTALRRARSAPAAAIAARVAGQTRNITVDPRLFKKRRL
RSPRVLFSTQPPREAADTQDLDFEVGGAAPFNRTHRSKRDDDDKSSSHPIFHRGEFSVCDSVSV
WVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRDPNPVDSGCRGIDSKHWNSYCTTT
HTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAVR

[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.



A B

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.

Nerve Growth Factor (NGF) is the archetypal neurotrophin of a family of polypeptides. It has long occupied a critical role in developmental and adult neurobiology for its many important regulatory functions on the survival, growth and differentiation of nerve cells in the peripheral and central nervous system. A functional binding ELISA assay was conducted to detect the interaction of recombinant human NGF and recombinant human CASP3. Briefly, NGF was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to CASP3-coated microtiter wells and incubated for 1h at 37℃. 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 for 1h at 37℃, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant human NGF and recombinant human CASP3 was shown in Figure 1, the EC50 for this effect is 0.08 ug/mL.

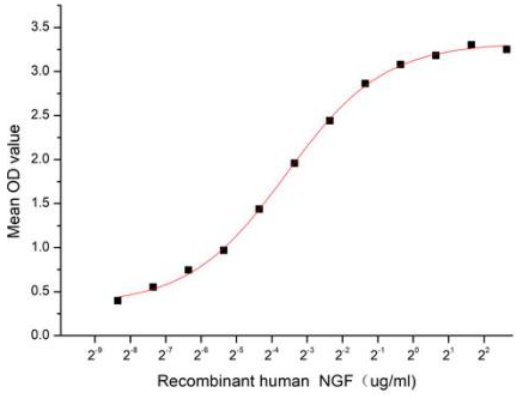


Figure 2. The binding activity of recombinant human NGF and recombinant human CASP3

[IDENTIFICATION]

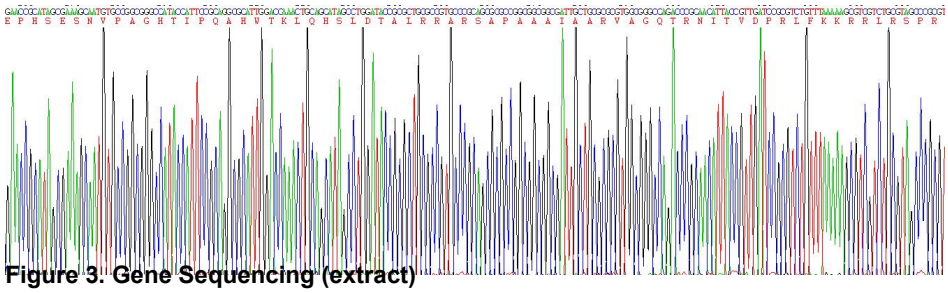


Figure 3. Gene Sequencing (extract)

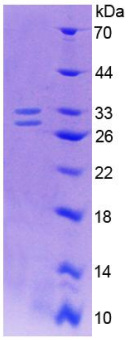


Figure 4. SDS-PAGE

Sample: Active recombinant NGF, Human

[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.