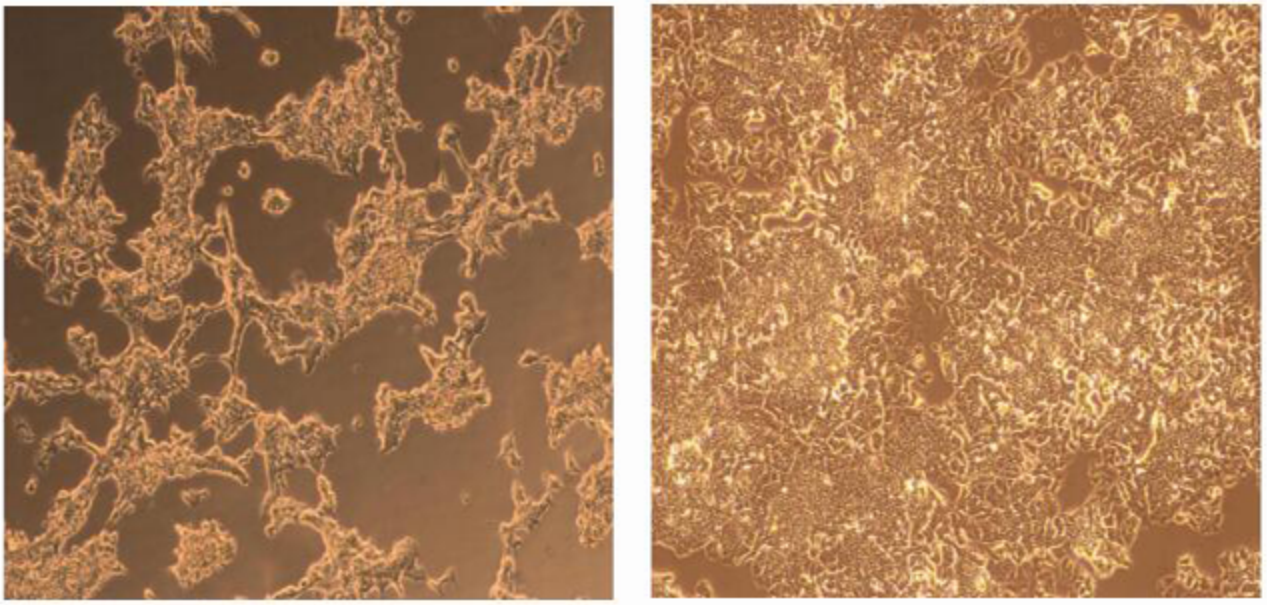
Active Nerve Growth Factor (NGF) Instruction Manual

SBPA068Hu01

Homo sapiens (Human)

Buffer Formulation	20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose and Proclin300.
Traits	Freeze-dried powder
Purity	> 97%
Isoelectric Point	10.1
Applications	Cell culture; Activity Assays.

ACTIVITY TEST



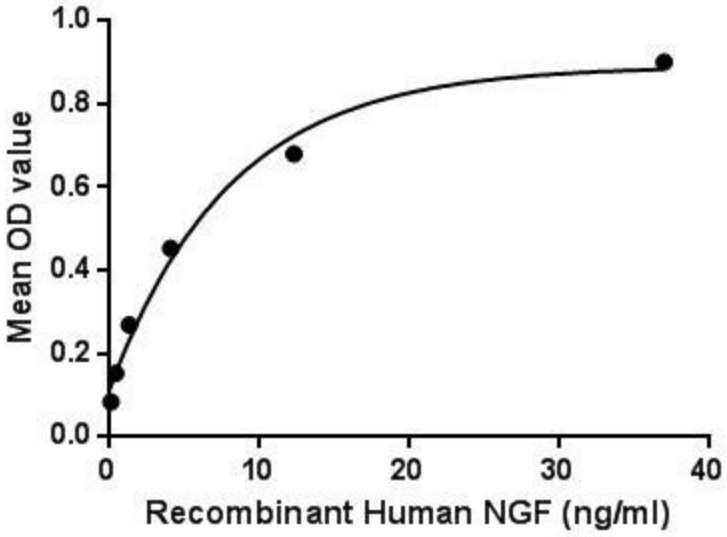
A B

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 a neurotrophic factor and neuropeptide primarily involved in the regulation of growth, maintenance, proliferation, and survival of certain target neurons. As reported, 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 polylysine or collagen coated plates. When cells reached log phase growth , fresh medium was added together with 10ng/mL of NGF, then cells were observed by inverted microscope everyday. Cell division ceasing and differentiation of PC12 cells after incubation with NGF (10ng/mL) for 6 days was shown in Figure1.Control group which received no NGF displayed no neurite outgrowth and cells multiply rapidly.



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 100μL were then transferred to a2M-coated microtiter wells and incubated for 2h 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, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL 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. The binding activity of NGF with a2M

USAGE

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

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

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.

Image

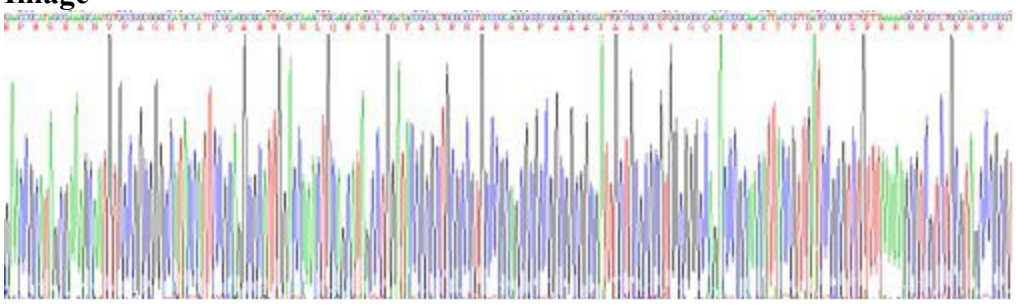


Figure. SDS-PAGE

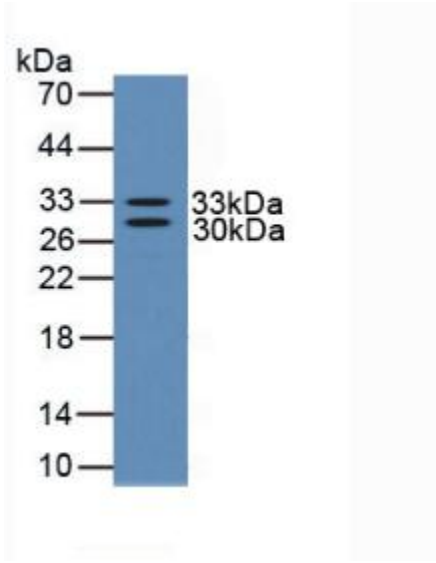


Figure. Western Blot; Sample: APA105Hu01; Antibody: PAA105Hu01

[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.