

Active Nesfatin 1 (NES1) Instruction Manual

SBPA106Ra01

Rattus norvegicus (Rat)

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%

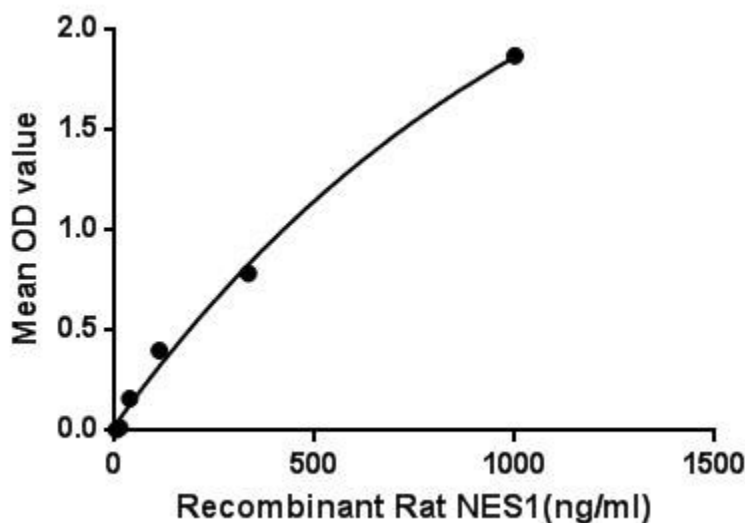
Isoelectric Point

5.8

Applications

Cell culture; Activity Assays.

ACTIVITY TEST



Nucb2 (Nucleobindin-2), a calcium-binding protein, is further cleaved into NES1 (Nesfatin-1). NES1 is an anorexigenic peptide and seems to participate in hypothalamic pathways regulating food intake and energy homeostasis, acting in a leptin-independent manner. GADD45A (Growth arrest and DNA damage-inducible protein GADD45 alpha) has been identified as an interactor of Nucb2. Besides, Growth Arrest And DNA Damage Inducible Protein Beta (GADD45b) has been identified as an interactor of NES1, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat NES1 and recombinant rat GADD45b. Briefly, NES1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µL were then transferred to GADD45b-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-NES1 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°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of NES1 and GADD45b was shown in Figure 1, and this effect was in a dose dependent manner.

Figure. The binding activity of NES1 with GADD45b.

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.

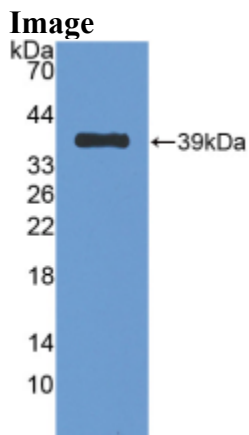


Figure. SDS-PAGE

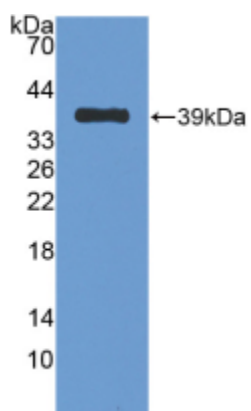


Figure. Western Blot

[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.