APA242Mu01 100μg
Active Nesfatin 1 (NES1)
Organism Species: Mus musculus (Mouse)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Pro26~Leu106
Tags: Two N-terminal Tags, His-tag and GST-tag
Purity: >98%
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.8
Predicted Molecular Mass: 39.5kDa
Accurate Molecular Mass: 39kDa as determined by SDS-PAGE reducing conditions.

[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 2-8ºC 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]

PIDVD KTKVHNVEPV ESARIEPPDT GLYYDEYLKQ VIEVLETDPH FREKLQKADI EEIRSGRLSQ ELDLVSHKVR TRLDEL

[ACTIVITY]

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, rat GADD45A shares similarities with mouse NES1 in amino acid sequence with the identity of 98%. Thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse NES1 and recombinant rat GADD45A. Briefly, NES1 were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100uL NES1 were then transferred to GADD45A-coated microtiter wells and incubated for 2h at 37℃. 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℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of NES1 and GADD45A was shown in Figure 1, and this effect was in a dose dependent manner.

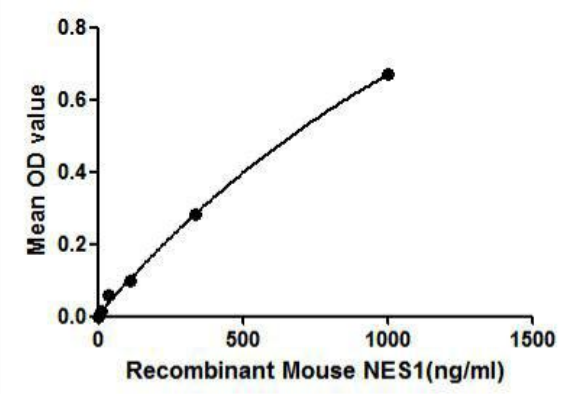

Figure 1. The binding activity of NES1 with GADD45A.

[IDENTIFICATION]

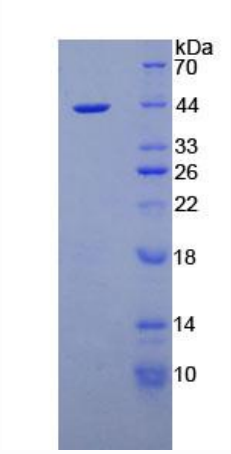

Figure 2. SDS-PAGE

Sample: Active recombinant NES1, Mouse

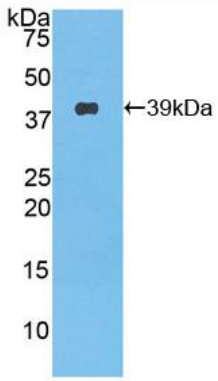

Figure 3. Western Blot

Sample: Recombinant NES1, Mouse;

Antibody: Rabbit Anti-Mouse NES1 Ab (PAA242Mu01)