

**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

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1st 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 2-8°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 ]**

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°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 GADD45A was shown in Figure 1, and this effect was in a dose dependent manner.

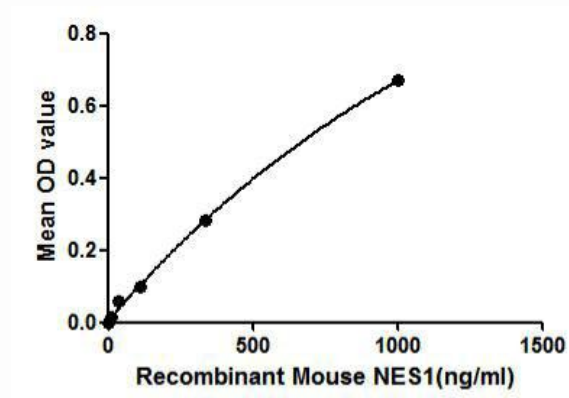


Figure 1. The binding activity of NES1 with GADD45A.

## [ IDENTIFICATION ]

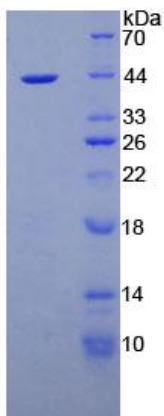
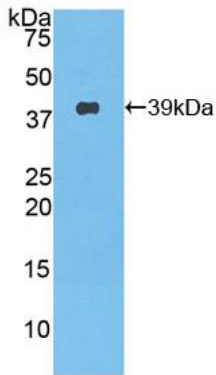


Figure 2. SDS-PAGE

Sample: Active recombinant NES1, Mouse



**Figure 3. Western Blot**

**Sample: Recombinant NES1, Mouse;**

**Antibody: Rabbit Anti-Mouse NES1 Ab (PAA242Mu01)**