

APE675Hu01 100µg

Active Active Regenerating Islet Derived Protein 3 Alpha (REG3a)

Organism Species: *Homo sapiens* (Human)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Cys40~Val170

Tags: N-terminal His-tag

Purity: >95%

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: 7.8

Predicted Molecular Mass: 16.0kDa

Accurate Molecular Mass: 15kDa 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]

CYALFLSPKS WTDADLACQK RPSGNLVSVL SGAEGSFVSS LVKSIGNSYS
YVWIGLHDPT QGTEPNGEGW EWSSSDVMNY FAWERNPSTI SSPGHCASLS
RSTAFLRWKD YNCNVRLPYV

[ACTIVITY]

Glial cell-derived neurotrophic factor (GDNF) is a small protein that potently promotes the survival of many types of neurons. This protein was shown to promote the survival and differentiation of dopaminergic neurons in culture, and was able to prevent apoptosis of motor neurons induced by axotomy. GDNF is processed to a mature secreted form that exists as a homodimer. The mature form of the protein is a ligand for the product of the RET (rearranged during transfection) protooncogene. Besides, Glial Cell Line Derived Neurotrophic Factor Receptor Alpha 2 (GFRa2) has been identified as an interactor of GDNF, thus a binding ELISA assay was conducted to detect the interaction of recombinant human GDNF and recombinant human GFRa2. Briefly, GDNF were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to GFRa2-coated microtiter wells and incubated for 2h at 37 °C . Wells were washed with PBST and incubated for 1h with anti-GDNF 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 GDNF and GFRa2 was shown in Figure 1, and this effect was in a dose dependent manner.

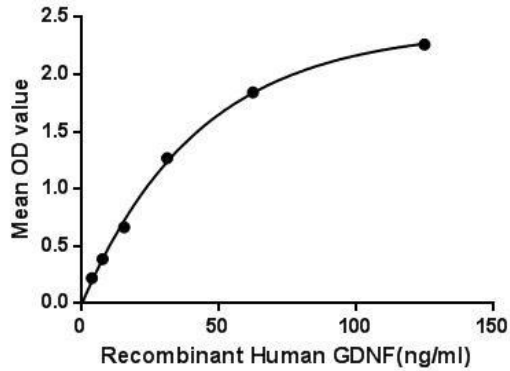


Figure 1. The binding activity of GDNF with GFRa2.

[IDENTIFICATION]

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TTCGTTGAAATGGACCTGGCTGCTAGTCTGTGGTTCGAGTTCGGCGAGGAGCCGCTCCAGGAGCCCTCTCGAATGGCGCTGCCTGGCTGGAGCCTCCTGCCTGAGCGAGCCAGCAGCAGCTGCTGCTGCTGG
CPKGSKATGSHCTAIFLSPKSNITDADLACQKRPFGMLVSYLSGAEGSPFVSSLVKRSIGNSYSYV#TGLHDPTQGTETPHGEVETWSSSDVM
  
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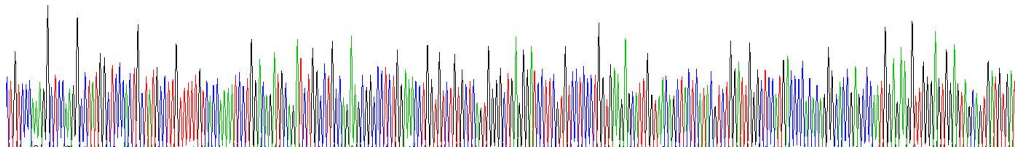


Figure 2. Gene Sequencing (extract)

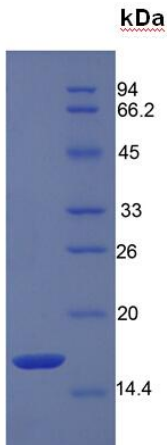


Figure 3. SDS-PAGE

Sample: Active recombinant REG3a, Human

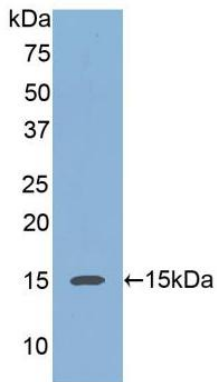


Figure 4. Western Blot

Sample: Recombinant REG3a, Human;

Antibody: Rabbit Anti-Human REG3a Ab (PAE675Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.