APD396Mu01 10μg
Active Proenkephalin (PENK)
Organism Species: *Mus musculus (Mouse)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Leu50~Phe268
Tags: His and TrxA Tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose .
Original Concentration: 100μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.3
Predicted Molecular Mass: 43.6kDa
Accurate Molecular Mass: 50kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in ddH_2O to a concentration of 0.1-0.5 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.

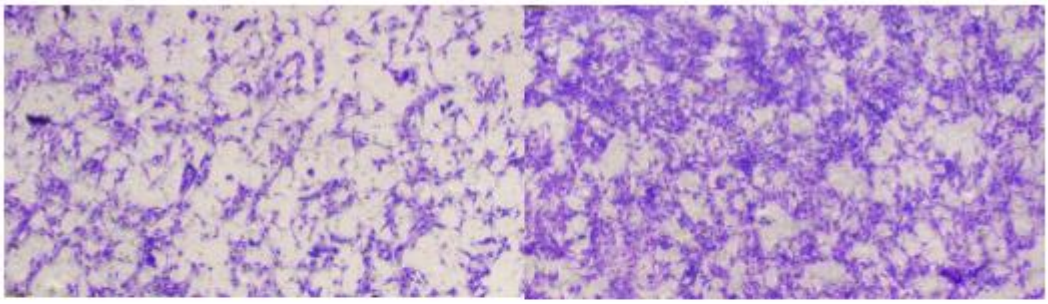
[SEQUENCE]

L
ECEGQLPSFK IWETCKDLLQ VSRPEFPWDN IDMYKDSSKQ DESHLLAKKY
GGFMKRYGGF MKKMDELYPM EPEEEANGGE ILAKRYGGFM KKDADEGDTL
ANSSDLLKEL LGTGDNRAKD SHQQESTNND EDMSKRYGGF MRSLKRSPQL
EDEAKELQKR YGGFMRRVGR PEWWMDYQKR YGGFLKRFAE SLPSDEEGEN
YSKEVPEIEK RYGGFMRF

[ACTIVITY]

Proenkephalin (PENK) is a protein encoded by the PENK gene. It is initially synthesized as a preproprotein and undergoes proteolytic processing to generate multiple products. As PENK has the function of inhibit the migration cells， we measure the activity of recombinant mouse PENK by the ability of the protein to inhibit the migration of U2OS cells. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the inhibition of chemotactic effect of recombinant mouse PENK on U2OS cell line. Briefly, U2OS cells were seeded into the upper chambers and PENK was added in lower chamber with a polycarbonate filter (8 μm pore size) used to separate the two compartments.

After incubation at 37℃ with 5% CO_2 for 2h, the filter was removed, then cells in low chamber were observed by inverted microscope at low magnification (×10) and the number of migrated cells were counted randomly (five fields for each filter). Result shows MIP-3α is able to induce migration of U2OS cells. The migrated U2OS cells in low chamber at low magnification (×10) were shown in Figure 1.



A B

Figure.1 The inhibition of chemotactic effect of recombinant mouse PENK on U2OS cells

(A) U2OS cells were seeded into the upper chambers and 2.5 μg/mL PENK was added in lower chamber, then cells in lower chamber were observed at low magnification (×10) after incubation for 2h;
(B) U2OS cells were seeded into the upper chambers without PENK was added in lower chamber, then cells in lower chamber were observed at low magnification (×10) after incubation for 2h.

[IDENTIFICATION]

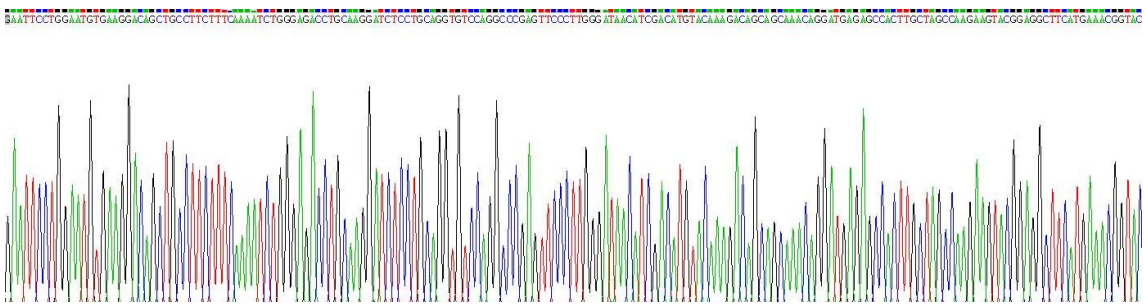


Figure 2. Gene Sequencing (extract)

Figure 3. SDS-PAGE

Sample: Active recombinant PENK, Mouse

[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.