

APE375Hu01 100μg

Active Lemur Tyrosine Kinase 3 (LMTK3)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

#### [PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Leu133~Leu411

Tags: Two N-terminal Tags, His-tag and SUMO-tag

**Purity: >95%** 

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA,

1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300. **Applications:** Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.3

Predicted Molecular Mass: 45.9kDa

Accurate Molecular Mass: 46kDa as determined by SDS-PAGE reducing conditions.

### [ <u>USAGE</u> ]

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]

LSYLQEIG SGWFGKVILG

EIFSDYTPAQ VVVKELRASA GPLEQRKFIS EAQPYRSLQH PNVLQCLGLC VETLPFLLIM EFCQLGDLKR YLRAQRPPEG LSPELPPRDL RTLQRMGLEI ARGLAHLHSH NYVHSDLALR NCLLTSDLTV RIGDYGLAHS NYKEDYYLTP ERLWIPLRWA APELLGELHG TFMVVDQSRE SNIWSLGVTL WELFEFGAQP YRHLSDEEVL AFVVRQQHVK LARPRLKLPY ADYWYDILQS CWRPPAQRPS ASDLQLQLTY L

#### [ACTIVITY]

Mechanism: Lemur tyrosine kinase 3 (LMTK3), a member of the receptor tyrosine kinase (RTK) family, is implicated in breast cancer growth and endocrine resistance. It has been reported that LMTK3 promotes cell invasion, motility, and migration of the MCF-7 breast cancer cell line. To test the bioactivity of LMTK3, MCF-7 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of LMTK3. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10  $\mu$ L of CCK-8 solution was added to each well of the plate, then measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37°C.

Cell proliferation of MCF-7 cells after incubation with LMTK3 for 72h observed by inverted microscope was shown in Figure 1.

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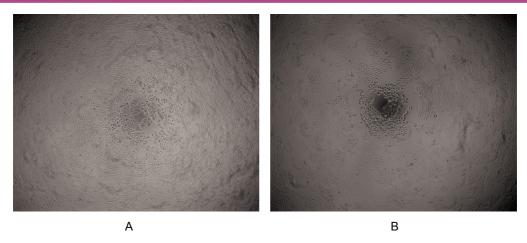


Figure 1. Cell proliferation of MCF-7 cells after stimulated with LMTK3.

- (A) MCF-7 cells cultured in serum-free DMEM, stimulated with 10ng/mL LMTK3 for 72h;
- (B) Unstimulated MCF-7 cells cultured in serum-free DMEM for 72h.

Cell proliferation was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with various concentrations of LMTK3 for 72h. The dose-effect curve of LMTK3 was shown in Figure 2. It was obvious that LMTK3 significantly promoted cell proliferation of MCF-7 cells. The ED50 for this effect is approximately 6.14~50ng/mL.

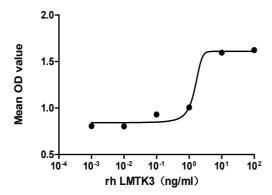


Figure 2. The dose-effect curve of LMTK3 on MCF-7 cells

## [ IDENTIFICATION ]

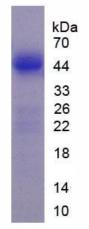


Figure 3. SDS-PAGE

Sample: Active recombinant LMTK3, Human

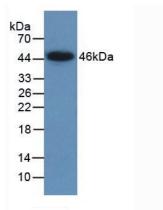


Figure 4. Western Blot

Sample: Recombinant LMTK3, Human;

Antibody: Rabbit Anti-Human LMTK3 Ab (PAE375Hu01)

#### [ 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.