

APB091Mu61 100µg
Active Alkaline Phosphatase, Tissue-nonspecific (ALPL)
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: Eukaryotic expression.

Host: 293F cell

Residues: Phe18~Gly503

Tags: N-terminal His-tag

Purity: >90%

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

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.9

Predicted Molecular Mass: 55.2kDa

Accurate Molecular Mass: 70kDa 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 10mM PBS (pH7.4) 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.

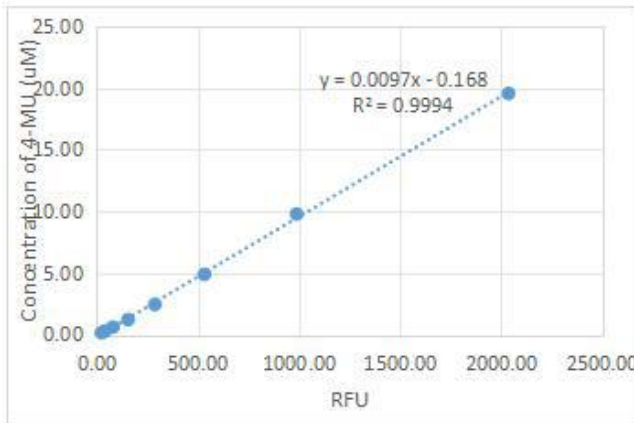
[SEQUENCE]

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DAGKSVGIVTTTRVNHATPSAAYAHSADRDWYSDNEMPPEALSQGCKDIAYQLMHNKIDIDVIMGGGRK  
YMYPKNRTDVEYELDEKARGTRLDGLDLISIWKSFKPRHKHSHYVWNRTPELLALDPSRVDYLLGLFEPG  
DMQYELNRNNTDPSLSEMVEVALRILTKNLKGFLLVEGGRIDHGHHEGKAKQALHEAVEMDQAIGKA  
GAMTSQKDTLTVVTADHSHVFTFGGYTPRGNSIFGLAPMVSDTDKKPFTAILYGNGPGYKVVDGERENV  
SMVDYAHNNYQAQSAVPLRHETHGGEDVAVFAKGPMAHLLHGVHEQNYIPHVMAYASCIGANLDHCAWA  
GSG
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[ACTIVITY]

Four distinct genes encode alkaline phosphatases (APs) in humans. The ALPL gene encodes the liver/bone/kidney isozyme, also known as the tissue-nonspecific AP (TNAP). In comparison, ALPI, ALPP and ALPPL2 encode intestinal, placental and placental-like or germ cell APs, respectively. The serum levels of human APs are useful tumor markers. There are many mutations in the ALPL gene, leading to different forms of hypophosphatasia, characterized by poorly mineralized cartilage and bones. The native ALPL is a glycosylated homodimer attached to the membrane through a GPI-anchor. The activity assay of recombinant mouse ALPL was measured by its ability to cleave a peptide

substrate, 4-Methumbelliferly phosphate. The reaction was performed in 50 mM Tris, 1 mM MgCl₂, pH 9.0 (assay buffer), ainitiated by addition 50 μ l of 0.3 ug/ml ALPL (diluted by assay buffer) to 50 μL of 50 uM substrate. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively, in kinetic mode for 5 minutes. The specific activity of recombinant mouse ALPL is >11000 pmol/min/μg.



RFU	4-MU (uM)
2035.62	19.53
987.32	9.77
531.52	4.88
286.52	2.44
154.42	1.22
79.52	0.61
41.54	0.31
22.43	0.15

Figure 1. The standard curve of 4-Methylumbelliferone

One unit of enzyme activity is defined as the 1 μg of enzyme required to convert 1 pmol of 4-Methumbelliferly phosphate to 4-Methylumbelliferone in 1 min.

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\Delta\text{RFU} * F}{T * N}$$

ΔRFU=Adjusted for Substrate Blank

F=Conversion Factor (convert from standard curve of 4-Methylumbelliferone (4-MU))

T= Time

N=Amount of enzyme

[IDENTIFICATION]

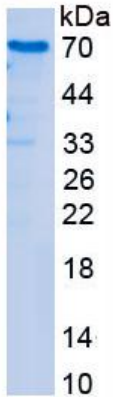


Figure 2. SDS-PAGE

Sample: Active recombinant ALPL, 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.