



Recombinant Mouse Alkaline Phosphatase/ALPL

Catalog Number: 2910-AP

Background

Several distinct genes encode alkaline phosphatases (APs) in mice with different tissue-specific expression patterns. The *Alpl* gene, also known as *Akp2*, encodes the liver/bone/kidney isozyme, also known as the tissue-nonspecific AP (TNAP) (1). The *Alpl* gene is a key regulator of bone mineralization in mice (2). A variety of mutations in the human ALPL gene leads to different forms of hypophosphatasia, characterized by poorly mineralized cartilage and bones (3). The native ALPL is a glycosylated homodimer attached to the membrane through a GPI-anchor. The C-terminal pro peptide (residues 504 to 524) is not present in the mature form.

References:

1. Terao, M. and B. Mintz (1987) Proc. Natl. Acad. Sci. USA 84:7051.
2. Hessele, L. *et al.* (2002) Proc. Natl. Acad. Sci. USA 99:9445.
3. Di Mauro, S. *et al.* (2002) J. Bone Miner. Res. 17:1383.

Description

Source	Murine myeloma cell line, NS0-derived Phe18 - Gly503, with a C-terminal 6-His tag Accession # P09242
N-terminal Sequence Analysis	Phe18
Predicted Molecular Mass	54 kDa

Specifications

SDS-PAGE	77 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl phosphate (4-MUP). The specific activity is > 40,000 pmoles/min/μg, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Preparation and Storage

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Do not freeze. <ul style="list-style-type: none">● 3 months from date of receipt, 2 to 8 °C as supplied.● 1 month, 2 to 8 °C under sterile conditions after opening.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 1 mM MgCl₂, pH 9.0
- Recombinant mouse Alkaline Phosphatase/ALPL/TNAP (mTNAP) (R&D Systems, Catalog # 2910-AP)
- Substrate: 4-Methumbelliferlyphosphate (4-MUP) (Calbiochem, Catalog # 474431)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute mTNAP to 0.02 ng/μL in Assay Buffer.
2. Dilute Substrate to 50 μM in Assay Buffer.
3. Load 50 μL of 0.02 ng/μL mTNAP into a plate, and start the reaction by adding 50 μL of 50 μM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of Substrate.
4. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmole/RFU)}}{\text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Methumbelliferone (Sigma, Catalog # 69580).

Final Assay Conditions Per Well

- mTNAP: 0.001 μg
- Substrate: 25 μM

9/30/2009

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NOT FOR USE IN HUMANS.