Recombinant Human Alkaline Phosphatase/ALPL

Catalog Number: 2909-AP

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Leu18-Ser502, with a C-terminal 6-His tag Accession # P05186
N-terminal Sequence Analysis	Leu18
Predicted Molecular Mass	54 kDa

SPECIFICATIONS

SDS-PAGE	65-75 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic substrate, 4-Methylumbelliferyl phosphate (4-MUP). The specific activity is >30,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES, NaCl, $MgCl_2$ and $ZnCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 1 mM $MgCl_2$, pH 9.0
- Recombinant Human Alkaline Phosphatase/ALPL/TNAP (rhTNAP) (Catalog # 2909-AP)
- Substrate: 4-Methumbelliferly phosphate (4-MUP) (Calbiochem, Catalog # 474431), 10 mM in deionized water
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhTNAP to 0.01 ng/µL in Assay Buffer.
2. Dilute Substrate 50 µM in Assay Buffer.
3. In a plate load 50 µL of 0.01 ng/µL rhTNAP to wells. Include a Substrate Blank of 50 µL of Assay Buffer.
4. Start the reaction by adding 50 µL of 50 µM Substrate to wells.
5. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively in kinetic mode for 5 minutes.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Methumbelliferone (Sigma, Catalog # M1381).

Final Assay Conditions

Per Well:

- rhTNAP: 0.0005 µg
- Substrate: 25 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Four distinct genes encode alkaline phosphatases (APs) in humans (1). 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 (2). There are many mutations in the ALPL gene, leading 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 503 to 524) is not present in the mature form.

References:

1. Le Du, M.-H. and J.L. Millan (2002) J. Biol. Chem. **277**:49808.
2. Millan, J.L. and W.H. Fishman (1995) Crit. Rev. Clin. Lab. Sci. **32**:1.
3. Di Mauro, S. *et al.* (2002) J. Bone Miner. Res. **17**:1383.

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