

DESCRIPTION

Source *Spodoptera frugiperda*, Sf 21 (baculovirus)-derived
Gln42-Trp749, with an N-terminal 6-His tag
Accession # AAC24487

N-terminal Sequence Analysis His & Leu47

Predicted Molecular Mass 83 kDa

SPECIFICATIONS

SDS-PAGE 68-85 kDa, reducing conditions

Activity Measured by its ability to cleave a fluorogenic peptide substrate, Mca-YVADAPK.
The specific activity is >125 pmol/min/μg, as measured under the described conditions.

Endotoxin Level <0.01 EU per 1 μg of the protein by the LAL method.

Purity >95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

Formulation Supplied as a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM MES, 250 mM NaCl, pH 6.0
 - Recombinant Human PHEX (rhPHEX) (Catalog # 8090-ZN)
 - Substrate: Mca-YVADAPK(Dnp)-OH (Catalog # ES007)
 - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
 - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rhPHEX to 4 ng/μL in Assay Buffer.
 2. Dilute Substrate to 100 μM in Assay Buffer.
 3. Load 50 μL of 4 ng/μL rhPHEX into a plate, and start the reaction by adding 50 μL of 100 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 320 nm and 405 nm, respectively in kinetic mode for 5 minutes.
 5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

- Final Assay Conditions**
- Per Well:
- rhPHEX: 0.2 μg
 - Substrate: 50 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

PHEX (Phosphate-regulating gene with homology to Endopeptidases on the X-chromosome; also known as metallopeptidase homolog PEX, vitamin D resistant hypophosphatemic rickets protein and X-linked hypophosphatemia protein/HYP) is a monomeric, zinc-dependent transmembrane member of the metallopeptidase M13 family of enzymes (1-6). Members of the M13 family are membrane-bound and characterized by the presence of a catalytic domain that consists of an ExxAG (or D) endopeptidase sequence, coupled to a canonical Zn-binding HEXxH motif (1, 3). PHEX expression is limited to a small number of cell types, including mature osteoblasts (7, 8), osteocytes (8, 9), and odontoblasts (but not ameloblasts) (7, 8). PHEX appears to have a material effect on mineralization. Typically, mineralization occurs when inorganic phosphate interacts with calcium to form hydroxyapatite. These crystals are deposited on the surface of either collagen I or DMP1 in bone matrix, and serve as nucleation sites for additional mineral deposition (10, 11). At select timepoints, osteocytes will downregulate the rate of mineralization, principally to insure that space exists between cell processes and calcified matrix. This is accomplished through the secretion of SIBLING family molecules (OPN; MEPE) that possess RGD and ASARM (Acidic Serine-and-Aspartate-Rich Motif) sequences. When cathepsin-like molecules are available, SIBLING molecules are cleaved, releasing ASARMS. If phosphorylated, these ASARMS bind to naked nucleation sites and terminate the growth of mineral. PHEX has a high affinity for ASARMS, and will cleave these fragments into smaller fragments, thus protecting the nucleation centers and promoting mineralization (12-14). In addition, PHEX will also bind the ASARM sequence in full-length SIBLING molecules (such as MEPE), possibly protecting them from proteolysis (15, 16). This blocks the generation of ASARMS that serve as antagonists to mineralization (15). Human PHEX is a 90-100 kDa, 749 amino acid (aa) type II transmembrane glycoprotein (3, 8, 9, 17). It contains a 20 aa cytoplasmic segment and a 708 aa extracellular region (aa 42-749) that possesses a lengthy catalytic domain (aa 67-741). Multiple point mutations are noted that adversely affect PHEX activity. There is one potential isoform variant that shows a six aa substitution for aa 1-293. Full-length human and mouse PHEX share 96% aa sequence identity.

References:

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