Recombinant Human ACP6

Catalog Number: 7766-AP

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Glu33-Glu428, with a C-terminal 6-His tag Accession # BAA91310
N-terminal Sequence Analysis	Glu33
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	43-45 kDa, reducing conditions
Activity	Measured by its ability to cleave a substrate, p-Nitrophenyl phosphate (pNPP). The specific activity is >1800 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 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 Sodium acetate, 0.05% Triton® X-100, pH 5.0
- Recombinant Human ACP6 (rhACP6) (Catalog # 7766-AP)
- Substrate: p-Nitrophenyl phosphate (Sigma, Catalog # N2765), 10 mM stock in deionized water
- NaOH, 0.2 M in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhACP6 to 0.8 µg/mL in Assay buffer.
2. Dilute Substrate to 2 mM in Assay buffer.
3. In a plate, load 50 µL of 0.8 µg/mL rhACP6, and start the reaction by adding 50 µL of 2 mM Substrate. Include a Substrate Blank containing 50 µL of Assay buffer and 50 µL of 2 mM Substrate.
4. Incubate plate at room temperature for 30 minutes in the dark.
5. Add 100 µL of 0.2 M NaOH to all wells to stop the reaction and develop the color.
6. Read plate at 410 nm (absorbance) in endpoint mode.
7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmol/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard p-Nitrophenol (Sigma, Catalog # 241326).

Final Assay Conditions

Per Well:

- rhACP6: 0.04 µg
- pNPP: 0.5 mM

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

Lysophosphatidic Acid Phosphatase Type 6 (ACP6) is a membrane-bound protein that belongs to the Histidine Acid Phosphatase Family. ACP6 is expressed in kidney, heart, small intestine, muscle, liver, prostate, testis, ovary, and colon tissues (1, 2). Its function is to hydrolyze lysophosphatidic acid (LPA) to form monoacylglycerol. LPA is one of the simplest of all known phospholipids which has a variety of biological functions including cell proliferation, migration, and survival (3). LPA is found at high levels in serum but also present in saliva, follicular fluid, seminal plasma and malignant effusions and is implicated in many cancers including ovarian, breast and prostate (3-5).

References:

1. Hiroyama, M. and T. Takenawa (1999) J. Biol. Chem. **274**:29172.
2. Takayama I. *et al.* (2002) Gut **50**:790.
3. Mills, G. and W. Moolenaar (2003) Nature **3**:582.
4. Xu, Y. *et al.* (1995) Biochem J. **309**:933.
5. Xie, Y. *et al.* (2002) J. Biol. Chem. **277**:32516.

PRODUCT SPECIFIC NOTICES

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449