Recombinant Human PLA2G4A

Catalog Number: 6659-PL

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human PLA2G4A protein Met1-Ala749, with a C-terminal 6-His tag Accession # NP_077734
N-terminal Sequence Analysis	Ser2
Predicted Molecular Mass	86 kDa

SPECIFICATIONS

SDS-PAGE	85-95 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze 1-Hexadecanoyl-2-(1-pyrene-decanoyl)-*sn*-glycero-3-phosphocholine. The specific activity is >15 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	>80%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, TCEP,Brij-35 and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 500 mM NaCl, 1 mg/mL BSA, pH 8.5
- Substrate Buffer: 50 mM Tris, 500 mM NaCl, 20 mM $CaCl_2$, 1 mg/mL BSA, pH 8.5
- Recombinant Human PLA2G4A (rhPLA2G4A) (Catalog # 6659-PL)
- Substrate: 1-Hexadecanoyl-2-(1-Pyrenedecanoyl)-sn-Glycero-3-Phosphocholine (Invitrogen, Catalog # H361), Dilute to 2 mM in DMSO, then dilute to a final stock concentration of 400 µM in ethanol
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Thaw Substrate at 37 °C for five minutes. Mix well.
2. Dilute rhPLA2G4A to 2 µg/mL in Assay Buffer.
3. Dilute Substrate to 10 µM in Substrate Buffer.
4. Load 50 µL of 2 µg/mL rhPLA2G4A into a black well plate, and start the reaction by adding 50 µL of 10 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 10 µM Substrate.
5. Read at excitation and emission wavelengths of 345 nm and 395 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 1-pyrenedecanoic acid (Invitrogen, Catalog # P31).

Final Assay Conditions

Per Well:

- rhPLA2G4A: 0.10 µg
- Substrate: 5 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.

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BACKGROUND

Cytosolic Phospholipase A2a (PLA2G4A) belongs to the group IV phospholipase A2 family (1). These enzymes hydrolyze the ester bond at the second position (sn-2) of membrane glycerophospholipids. The group IV phospholipase A2 (PLA2G4) family is comprised of six intracellular enzymes (2). PLA2G4A has a preference for arachidonic acid in the sn-2 position of phospholipids indicating its involvement in the metabolism of eicosanoids. Its active site is characterized by a serine and aspartic acid dyad. The overall structural feature displays two domains, a lipid binding C2 domain and a catalytic a/b hydrolase domain. PLA2G4A translocation to the membrane and activation is facilitated by a calcium-ion dependent conformational change in its C2 domain. In addition, phosphorylation of serine residues in PLA2G4A by protein kinases may be another regulatory mechanism for promoting the release of arachidonic acid. The knockout mouse studies of this enzyme indicate that the enzyme may play a major role in inflammatory diseases. Genetic ablation or reduction of PLA2G4A protected human amyloid precursor protein (APP) related pathogenesis from a mouse model (3). Mouse model studies of brain and lung cancer indicates that PLA2G4A may play a key regulatory role in angiogenesis of these tumors (4).

References:

1. Burke, J. E. and E.A. Dennis (2009) J. Lipid Res. **50**:S237.
2. Ghosh, M. *et al.* (2006) Prog. Lipid Res. **45**:487.
3. Sanchez-Mejia, R.O. *et al.* (2008) Nat. Neuroscience **11**:1311.
4. Linkous, A.G. *et al.* (2010) J. Natl. Cancer Inst. **102**:1398.

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