Recombinant Human Leukotriene A4 Hydrolase/LTA4H

Catalog Number: 4008-ZN

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Pro2-Asp611, with a C-terminal 10-His tag Accession # P09960
N-terminal Sequence Analysis	Pro2
Predicted Molecular Mass	70 kDa

SPECIFICATIONS

SDS-PAGE	71 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Arg-7-amido-4-methylcoumarin (R-AMC). The specific activity is >70 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 Tris, NaCl, and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, pH 7.5 (TCN)
- Recombinant Human Leukotriene A4 Hydrolase/LTA4H (rhLTA4H) (Catalog # 4008-ZN)
- Substrate: H-Arg-AMC (Bachem, Catalog # I-1050 or ChemImpex, Catalog # 5859), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhLTA4H to 10 µg/mL in Assay Buffer.
2. Dilute Substrate to 800 µM in Assay Buffer.
3. Load 50 µL of 10 µg/mL rhLTA4H into a plate, and start the reaction by adding 50 µL of 800 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 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. 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 7-Amino, 4-Methyl Coumarin (AMC) (Sigma-Aldrich, Catalog # A9891).

Final Assay Conditions

Per Well:

- rhLTA4H: 0.5 µg
- Substrate: 400 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

Leukotrienes are a family of lipid mediators important in a variety of allergic and inflammatory reactions. Synthesized by leukocytes, these molecules are divided into two classes, the spasmogenic cysteinyl leukotrienes and LTB4, a classical chemoattractant (1). Encoded by the LTA4H gene, Leukotriene A4 Hydrolase catalyzes the conversion of unstable epoxide LTA4 to LTB4, which is the final and committed step in LTB4 biosynthesis. As a bifunctional zinc metalloenzyme, LTA4H also acts as an arginyl aminopeptidase (2). LTA4H is a drug target for anti-inflammation, and for cancer prevention and therapy (1, 3).

The mature chain of human LTA4H consists of 610 amino acids (residues 2-611). It is highly specific for LTA4, which also covalently modifies and inhibits the enzyme. The aminopeptidase activity is enhanced by monovalent anions (1). R&D Systems recombinant human LTA4H corresponds to the mature chain, and is characterized by its arginyl aminopeptidase activity.

References:

1. Haeggstrom, J.Z. (2006) J. Biol. Chem. **279**:50639.
2. Orning, L. *et al.* (1994) J. Biol. Chem. **269**:11269.
3. Chen, X. *et al.* (2004) Curr. Cancer Drug Targets **4**:267.

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