Recombinant Mouse ASAHL/ N-acylethanolamine-hydrolyzing Acid A

Catalog Number: 4886-AH

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Val33-Ser362, with a C-terminal 10-His tag Accession # AAH04572
N-terminal Sequence Analysis	Val33
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	50 kDa, 33 kDa and 19 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the substrate palmitoylethanolamide into palmitate and ethanolamine. The specific activity is >100 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	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M Sodium Acetate, 0.1% (v/v) NP-40 substitute (Fluka, Catalog # 74385), pH 4.0
- Recombinant Mouse ASAHL/N-acylethanolamine-hydrolyzing Acid A (rmASAHL) (Catalog # 4886-AH)
- Palmitoyl Ethanolamide (PEA) (Tocris, Catalog # 0879), 25 mM stock in dimethyl formamide
- Dithiothreitol (DTT) (Sigma, Catalog # D0632), 1 M stock in deionized water
- Sodium Hydroxide
- β-mercaptoethanol (Sigma, Catalog # M7154)
- o-phthaldialdehyde (o-PA) (Sigma, Catalog # P0657), 50 mg/mL 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 PEA to 50 µM in Assay Buffer. Dissolve 10 µL of 25 mM stock into 4.99 mL of Assay Buffer (Note: Preheat assay buffer to 37 °C and vortex for 30 seconds to completely solubilize the PEA).
2. Dilute rmASAHL to 1.25 µg/mL in Assay Buffer.
3. Set up reactions in 1.5 mL microtubes. Mix 200 µL of 50 µM PEA, 50 µL of 1.25 µg/mL rmASAHL, and 2.5 µL of 1 M DTT.
4. Incubate reaction tubes at 37 °C for 1 hour.
5. Prepare an o-PA solution. Mix 3.84 mL of 0.2 M Sodium Hydroxide, 4 µL β-mercaptoethanol, and 160 µL 50 mg/mL o-PA.
6. Add 250 µL of the o-PA mixture (step 5) to all reaction vials. Mix well and incubate at room temperature for 10 minutes.
7. Create a control vial by combining, in this order, 250 µL o-PA mixture (step 5), 50 µL of 1.25 µg/mL rmASAHL, and 200 µL of 50 µM PEA.
8. Load in a plate 200 µL in duplicate of reaction mixtures and control.
9. Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.
10. Calculate specific activity (Average duplicates):

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

*Adjusted for control

**Derived using calibration standard ethanolamine (Sigma, Catalog # E9508).

Final Assay Conditions

Per Well:

- rmASAHL: 0.025 µg
- PEA: 20 µM
- o-PA: 1 mg/mL

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

The mouse ASAHL gene encodes N-acylethanolamine-hydrolyzing Acid Amidase (NAAA), a fatty acid amidase with maximal activity at acidic pH (1). NAAA hydrolyzes a number of *N*-acyl ethanolamines, including *N*-myristoyl-, *N*-stearoyl-, *N*-oleoyl-, and *N*-arachidonoyl, but is most active against *N*-palmitoylethanolamine (2). NAAA is a member of the choloylglycine hydrolase family of enzymes, and is structurally similar to acid ceramidase (1). NAAA is both a lysosomal and a secreted enzyme, and like acid ceramidase, has been observed to be proteolytically processed during maturation (1). Through its amidase activity, ASAHL may play a role in the termination of the actions of a variety of N-acylethanolamides (3). NAAA can be distinguished from anandamide amidohydrolase by its lack of inhibition by methyl arachidonoyl fluorophosphonate (2).

References:

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2. Ueda, N. *et al.* (2001) J. Biol. Chem. **276**:35552.
3. Sun, Y. X. *et al.* (2005) Biochim. Biophys. Acta **1736**:211.

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