R&D SYSTEMS
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# Recombinant Human ASAHL/ N-acylethanolamine-hydrolyzing Acid A

Catalog Number: 4494-AH

## DESCRIPTION

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| **Source** | Chinese Hamster Ovary cell line, CHO-derived<br>Met1-Lys359 (Val151Ile), with a C-terminal 10-His tag<br>Accession # Q02083 |
| **N-terminal Sequence Analysis** | Ser29 |
| **Predicted Molecular Mass** | 39 kDa |

## SPECIFICATIONS

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| **SDS-PAGE** | 50 kDa, 33 kDa and 20 kDa, reducing conditions. |
| **Activity** | Measured by its ability to hydrolyze the substrate palmitoylethanolamide into palmitate and ethanolamine.<br>The specific activity is >300 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 silver stain. |
| **Formulation** | Supplied as a 0.2 µm filtered solution in MES, NaCl and Glycerol. See Certificate of Analysis for details. |

## Activity Assay Protocol

**Materials**

- Assay Buffer: 0.1 M NaOAc, 0.1% (v/v) NP-40 substitute (Fluka, Catalog # 74385), pH 4.0
- Recombinant Human ASAHL/N-acylethanolamine-hydrolyzing Acid A (rhASAHL) (Catalog # 4494-AH)
- Palmitoyl Ethanolamide (PEA) (Tocris, (Tocris, Catalog # 0879), 25 mM stock in dimethyl formamide
- Dithiothreitol (DTT) (Sigma, Catalog # D0632), 1 M stock in deionized water
- NaOH (Sigma, Catalog # 221465), 2 M stock in deionized water
- β-mercaptoethanol (Sigma, Catalog # M-7154)
- 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 by dissolving 10 µL of 25 mM stock in 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 rhASAHL to 1.25 µg/mL in Assay Buffer.
3. Combine 200 µL of 50 µM PEA, 50 µL of 1.25 µg/mL rhASAHL, and 2.5 µL of 1 M DTT.
4. Incubate reaction tubes at 37 °C for 1 hour.
5. Dilute NaOH to 0.2 M in deionized water.
6. Combine 3.84 mL of 0.2 M NaOH with 4 µL β-mercaptoethanol and 160 µL of 50 mg/mL o-PA.
7. Stop the reactions by adding 250 µL of the o-PA mixture (step 6) to all the vials and mix well.
8. Incubate at room temperature for 10 minutes.
9. Combine 250 µL of o-PA mixture, 50 µL of 1.25 µg/mL rhASAHL, and 200 µL of 50 µM PEA in this order for a control.
10. Load 200 µL of reaction mixtures and control in a plate.
11. Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.
12. 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:

- rhASAHL: 0.025 µg
- Palmitoyl Ethanolamide: 20 µM
- o-PA: 1 mg/mL

## PREPARATION AND STORAGE

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| **Shipping** | The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below. |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>• 6 months from date of receipt, -20 to -70 °C as supplied.<br>• 3 months, -20 to -70 °C under sterile conditions after opening. |



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## BACKGROUND

The human NAAA gene encodes N-acylethanolamine-hydrolyzing acid amidase, also known as ASAH-like protein (ASAHL), 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 cholylglycine hydrolase family of enzymes, and is structurally similar to acid ceramidase (3). NAAA is both a lysosomal and a secreted enzyme, and like acid ceramidase, has been observed to be proteolytically processed during maturation (3). NAAA can be distinguished from anandamide amidohydrolase by its lack of inhibition by methyl arachidonyl fluorophosphonate (2).

**References:**

1. Hong, S.B. *et al*. (1999) Genomics **62**:232.
2. Ueda, N. *et al*. (2001) J. Biol. Chem. **276**:35552.
3. Tsuboi, K. *et al*. (2005) J. Biol. Chem. **280**:11082.



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