Recombinant Human Dimethylarginine Dimethylaminohydrolase 1/DDAH1

Catalog Number: 6530-DA

DESCRIPTION

Source	*E. coli*-derived Ala2-Ser285, with an N-terminal Met and 6-His tag Accession # O94760
N-terminal Sequence Analysis	Inconclusive result, Met predicted. Protein identity confirmed by MS analysis of tryptic fragments.
Predicted Molecular Mass	32 kDa

SPECIFICATIONS

SDS-PAGE	38 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze asymmetric dimethylarginine to L-citrulline and dimethylamine. The specific activity is >75 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, NaCl, EDTA, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 5 mM 1,10-phenanthroline, 5 mM TCEP, pH 8.5
- 1,10-phenanthroline (Sigma, Catalog # 320056), 0.6 M stock in DMSO
- Tris(2-carboxyethyl)phosphine (TCEP) (Calbiochem, Catalog # 580560), 0.5 M in deionized water
- Recombinant Human Dimethylarginine Dimethylaminohydrolase 1/DDAH1 (rhDDAH1) (Catalog # 6530-DA)
- Asym-dimethylarginine (ADMA) (Sigma, Catalog # D4268), 100 mM stock in deionized water
- 2,3-Butanedione monoxime (DAMO) (Sigma, Catalog # B0753)
- Thiosemicarbazide (Sigma, Catalog # 89050)
- o-Phosphoric acid, 85% (Fisher, Catalog # A242)
- Sulfuric acid (Fisher, Catalog # A300)
- Ammonium iron (III) sulfate dodecahydrate (Sigma, Catalog # 221260)
- L-Citrulline (Sigma, Catalog #C7269), 20 mM stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare Color Developing Reagent A (80 mM DAMO, 2 mM Thiosemicarbazide) in deionized water.
2. Prepare Color Developing Reagent B (17.35% (v/v) Phosphoric Acid, 33.7% (v/v) Sulfuric Acid, 0.765 mg/mL Ammonium Iron Sulfate) in deionized water.
3. Dilute rhDDAH1 to 40 ng/µL in Assay Buffer.
4. Dilute ADMA to 20 mM in Assay Buffer.
5. Dilute 20 mM L-Citrulline stock to 1000 µM in Assay Buffer. This is the first point of the standard curve.
6. Continue standard curve by performing six one-half serial dilutions of the 1000 µM L-Citrulline in Assay Buffer. The standard curve has a range of 0.625 to 40 nmol per well.
7. Pipet 150 µL of each point of the standard curve into microtubes.
8. Combine 75 µL of 40 ng/µL rhDDAH1 with 75 µL of 20 mM ADMA in microtubes. Include a Substrate Blank containing Assay Buffer in place of rhDDAH1.
9. Incubate reaction, blank, and standard curve for 1 hour at 37 °C.
10. Prepare Color Developing Reagent C (1 part Reagent A: 3 parts Reagent B).
11. Add 600 µL of Color Developing Reagent C to reaction, blank, and standard curve tubes.
12. Heat the tubes at 95-100 °C in a heating block for 15 minutes.
13. Cool tubes at room temperature for 5 minutes.
14. Load 200 µL into wells.
15. Read plate at 540 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Product* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the L-Citrulline standard curve using linear fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Well:

- rhDDAH1: 0.8 µg
- ADMA: 2 mM

PREPARATION AND STORAGE

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.

- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Dimethylarginine Dimethylaminohydrolase (DDAH) metabolizes asymmetric dimethyl arginine (ADMA) to L-citrulline and dimethylamine, and N^G-monomethyl arginine (MMA) to L-citrulline and monomethylamine (1). Two members of the DDAH family have been identified in humans. DDAH1 is widely expressed, especially in liver and kidney. DDAH2 predominates in vascular endothelium and expressed selectively in kidney (2). It is also expressed in immune tissues including spleen, thymus, peripheral leukocytes, lymph nodes, and bone marrow. Over 90 % of endogenous ADMA is metabolized by DDAH with the remainder excreted (3). ADMA and MMA are endogenous inhibitors of nitric oxide synthase (NOS). Thus, enzymes of the DDAH family play a key role in vascular function through the turnover of methylated arginine (4). It has been observed that genetic variation in the DDAH1 and DDAH2 genes is significantly associated with serum ADMA levels (5).

References:

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4. Leiper, J. *et al.* (2007) Nat. Med. **13**:198.
5. Abhary, S. *et al.* (2010) PNAS PLoS One. **5**:e9462.

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