Recombinant Human D-Amino Acid Oxidase His-tag

Catalog Number: 10116-DO

DESCRIPTION

Source	*E. coli*-derived human D-Amino Acid Oxidase protein Arg2-Leu347 Accession # P14920-1 with N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	40 kDa

SPECIFICATIONS

SDS-PAGE	39 kDa, under reducing conditions
Activity	Measured by its ability to produce hydrogen peroxide during the oxidative deamination of D-amino acids. The specific activity is >1000 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 Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Buffer: 50 mM Sodium Phosphate, pH 8.0
- Flavin Adenine Dinucleotide (FAD) (Sigma, Catalog # F6625), 10 mM stock in deionized water
- Recombinant Human D-Amino Acid Oxidase His-tag (rhDAO) (Catalog # 10116-DO)
- Coupling Enzyme: Horseradish Peroxidase (HRP) (250-333 U/mg) (Sigma, Catalog # P8375), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- Substrate Component 1: D-Alanine (Sigma, Catalog # A7377), 500 mM stock in deionized water
- Substrate Component 2: Amplex Ultra Red (AUR) (Molecular Probes, Catalog # A36006), 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. Prepare Assay Buffer (50 mM Sodium phosphate, 5 µM FAD, pH 8.0) by adding 10 mM stock of FAD to Buffer right before use.
2. Dilute rhDAO to 2 ng/µL in Assay Buffer.
3. Dilute D-Alanine to 40 mM in Assay Buffer.
4. Combine equal volumes of 2 ng/µL rhDAO and 40 mM D-Alanine. Also, create a Substrate Blank by combining equal volumes of Assay Buffer and 40 mM D-Alanine.
5. Incubate the reactions for 10 minutes at 37 °C.
6. Prepare Substrate Mixture containing 2 units/mL HRP and 100 µM AUR in Assay Buffer.
7. Load 50 µL of the incubated reactions into the wells of a black well plate, and start the reaction by adding 50 µL of Substrate Mixture.
8. Read at excitation and emission wavelengths of 544 nm and 590 nm (top read), respectively in endpoint mode. Note: A cutoff must be set manually at a wavelength of 570 nm.
9. Calculate specific activity:

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

*Adjusted for Substrate Blank
**Derived using a fluorescent standard prepared by incubating 50 µM AUR, 1 unit/mL HRP, 10 mM D-Alanine, and a curve of Hydrogen Peroxide (Sigma, Catalog # H1009) in Assay Buffer. Use this oxidized AUR curve to determine the conversion factor.

Final Assay Conditions

Per Well:

- rhDAO: 0.05 µg
- D-Alanine: 10 mM
- HRP: 1 unit/mL
- AUR: 50 µM

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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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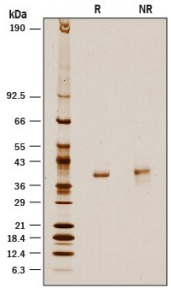
SDS-PAGE



1 µg/lane of Recombinant Human D-Amino Acid Oxidase His-tag (Catalog # 10116-DO) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing a band at 39 kDa.

BACKGROUND

D-amino acid oxidase (DAO) is an FAD-dependent enzyme that catalyzes the strict stereoselective oxidative deamination of D-amino acids. Human DAO is an 80 kDa stable homodimer of monomers in head to head conformation (1). Each monomer has two domains: a substrate-binding domain containing the active site and responsible for oligomerization and the FAD-binding domain (2). Mammalian DAO is primarily expressed in kidney, liver and brain, but also in small intestine and neutrophilic leukocytes (3). Human DAO differs in biophysical characteristics compared to other species due to its weakly bound cofactor, modulation by the regulatory protein pLG72 (4), and relatively low activity on its physiological substrates: D-serine, D-Cys, and D-DOPA (5). D-serine is a key neuromodulator and co-agonist of N-methyl-D-aspartate receptors (NMDAR), receptors that are critically involved in learning and memory. Abnormal levels of D-serine are associated with chronic neurodegeneration and misregulation of DAO. While many variants of DAO are implicated in neurological disease, a specific DAO gene mutation resulting in an enzyme with impaired activity causes dominantly inherited familial amyotrophic lateral sclerosis (ALS) (6). Only variants in the DAO gene are significantly associated with the clinical outcome of ALS patients (7). In Alzheimer's disease (AD), DAO expression is higher and correlates with severity of cognitive impairment (8) while inhibition resulted in improved cognitive function in AD patients (9). Likewise, as with AD, increased expression of DAO with concomitant decreased D-serine levels is found in schizophrenia with beneficial results observed upon therapeutic delivery of D-serine (10). DAO inhibition has also been shown to modulate chronic pain (11, 12). DAO is a pharmaceutical target for the treatment of neurological therapeutic disease and pain (13, 14).

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