Recombinant Human
Aldehyde Dehydrogenase 3-A1/ALDH3A1
Catalog Number: 6705-DH

DESCRIPTION

Source
E. coli-derived human Aldehyde Dehydrogenase 3-A1/ALDH3A1 protein
Ser2-His453, with an N-terminal Met and 6-His tag
Accession # P30838

N-terminal Sequence Analysis
Met

Predicted Molecular Mass
51 kDa

SPECIFICATIONS

SDS-PAGE
51-56 kDa, reducing conditions

Activity
Measured by the ability to catalyze the oxidation of 4-nitrobenzaldehyde.
The specific activity is >6000 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, Glycerol and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 50 mM Tris, 5 mM DTT, pH 9.0
- Recombinant Human Aldehyde Dehydrogenase 3-A1/ALDH3A1 (rhALDH3A1) (Catalog # 6705-DH)
- Nicotinamide adenine dinucleotide phosphate (NADP⁺) (Sigma, Catalog # N5755), 50 mM stock in deionized water
- 4-Nitrobenzaldehyde (4-NBA) (Sigma, Catalog # 72800), 200 mM stock in DMSO
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Dilute rhALDH3A1 to 4 ng/µL in Assay Buffer.
2. Dilute NADP⁺ to 2 mM in Assay Buffer.
3. Dilute 4-NBA to 4 mM in Assay Buffer.
4. Form Substrate Mixture by combining equal volumes of 2 mM NADP⁺ and 4 mM 4-NBA.
5. Load 50 µL of the 4 ng/µL rhALDH3A1 into the plate. Include a Substrate Blank containing 50 µL of Assay Buffer.
6. Start the reaction by adding 50 µL of Substrate Mixture to the wells.
7. Read plate at 340 nm (absorbance) in kinetic mode for 5 minutes.
8. Calculate specific activity:

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\text{Specific Activity (pmol/min/µg) = \frac{\text{Adjusted } V_{\text{max}} \times \text{OD/min} \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol ext. coeff}^{**} \times \text{path corr.*** (cm) \times amount of enzyme (µg)}}{\text{ext. coeff}^{**} (M^{-1} cm^{-1}) \times \text{path corr.*** (cm) \times amount of enzyme (µg)}}
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*Adjusted for Substrate Blank
**Using the extinction coefficient 6270 M⁻¹ cm⁻¹
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:
- rhALDH3A1: 0.2 µg
- NADP⁺: 0.5 mM
- 4-NBA: 1 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.
Aldehyde Dehydrogenases (ALDHs) are NAD(P)^+ dependent enzymes that catalyze the oxidation of endogeneously produced and exogeneous aldehydes to their corresponding acids (1). They are involved in the detoxification of alcohol-derived acetaldehyde and in the metabolism of corticosteroids, biogenic amines, neurotransmitters, and in lipid peroxidation. ALDH3A1 is also known as stomach aldehyde dehydrogenase. It exists as a homodimer, and prefers NADP^+ over NAD^+ as its co-factor (2). It preferentially oxidizes aromatic and medium-chain (6 carbons or more) saturated and unsaturated aldehyde substrates (3). The enzyme is highly expressed in stomach and cornea (2, 4). In the cornea, its proposed roles have been to absorb UV-light, reduce oxidative damage, maintain corneal refractive and transparency properties, and display chaperone-like activity (4, 5). It also has been identified as a lung cancer biomarker (6).

References: