Recombinant Human Dopamine β-Hydroxylase

Catalog Number: 7376-AO

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Met1-Gly617, with a C-terminal 6-His tag Accession # P09172
N-terminal Sequence Analysis	Leu37 & Ser40
Predicted Molecular Mass	66 kDa

SPECIFICATIONS

SDS-PAGE	65-85 kDa, reducing conditions
Activity	Measured by its ability to convert tyramine to octopamine. The specific activity is >7,000 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M NaOAc, 0.5 µM $CuCl_2$, pH 5.0
- Recombinant Human Dopamine β-Hydroxylase (rhDBH) (Catalog # 7376-AO)
- Substrate Component 1: Tyramine (Sigma, Catalog # T2879), 100 mM stock in deionized water
- Substrate Component 2: N,N-Dimethyl-p-phenylenediamine (DMPD) (Sigma, Catalog # D4139), 500 mM stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhDBH to 1 ng/µL in Assay Buffer.
2. Dilute both substrate components to 40 mM respectively in Assay Buffer.
3. Combine equivalent volumes of substrate components for substrate mixture.
4. Load 50 µL of the diluted rhDBH into a clear plate, and start the reaction by adding 50 µL of the Substrate mixture to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate Mixture without any rhDBH.
5. Read in kinetic mode for 5 minutes at an absorbance of 515 nm.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 5200 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhDBH: 0.050 µg
- Tyramine: 10 mM
- DMPD: 10 mM

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.

BACKGROUND

Dopamine β-Hydroxylase (DBH), also known as dopamine β-monooxygenase, belongs to the copper type II, ascorbate-dependent monooxygenase family. DBH is found within the neurosecretory vesicles of adrenal medullae and the large dense-cored synaptic vesicles of the sympathetic nervous system as both membrane-associated and soluble forms (1, 2). It catalyzes the conversion of dopamine to noradrenaline in sympathetic neurons, making it an important enzyme for catecholamine biosynthesis (3, 4). Mutations in the DBH gene that result in low DBH activity are a cause of noradrenaline deficiency (5). This recombinant human DBH is the secreted, soluble form.

References:

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3. Levin, E.Y. *et al.* (1960) J. Biol. Chem. **235**:2080.
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5. Kim, C.H. *et al.* (2002) Am. J. Med. Genet. **108**:140.

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