Recombinant Human ODC1 His-tag

Catalog Number: 10316-OD

DESCRIPTION

Source	*E. coli*-derived human ODC1 protein Asn2-Val461 Accession # P11926 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	49 kDa, under reducing conditions
Activity	Measured by its ability to convert ornithine to putrescine. The specific activity is >750 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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, NaCl and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.5
- Enzyme Buffer: 50 mM Tris, 0.1 mM EDTA, 0.1 mM Pyridoxal 5'-phosphate, 2.5 mM DTT, 0.1% Tween 80, pH 7.5
- Recombinant Human Ornithine Decarboxylase 1 (rhODC1) (Catalog # 10316-OD)
- Substrate: L-Ornithine monohydrochloride (Sigma, Catalog # O2375), 1 M stock in deionized water
- Cucurbit[6]uril Hydrate (CB6) (Sigma, Catalog # 94544), 150 µM stock in 50 mM Tris and 1 M HCl
- Trans-4-[4-(Dimethylamino)styryl]-1-methylpyridinium iodide (DSMI) (Sigma, Catalog # 336408), 40 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax M5 by Molecular Devices) or equivalent

Assay

1. Dilute CB6 and DSMI to 0.667 µM and 4 µM in Assay Buffer, respectively.
2. In a plate load 150 µL of diluted CB6/DSMI mixture and incubate for 10 minutes at room temperature in the dark.
3. Dilute rhODC1 to 0.1 µg/mL in Enzyme Buffer.
4. Dilute Substrate to 200 µM in Assay Buffer.
5. Add 25 µL of 0.1 µg/mL rhODC1 to wells containing the CB6/DSMI mixture, and start the reaction by adding 25 µL of 200 µM Substrate. Include a Substrate Blank containing 25 µL of Enzyme Buffer and 25 µL of 200 µM Substrate (along with 150 µL of CB6/DSMI mixture).
6. Read at excitation and emission wavelengths of 450 nm and 582 nm (top read), respectively, in kinetic mode for 5 minutes.
7. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard putrescine (Sigma, Catalog # 51799) in the presence of the CB6/DSMI mixture.

Final Assay Conditions

Per Well:

- rhODC1: 0.0025 µg
- CB6: 0.5 µM
- DSMI: 3 µM
- Substrate: 25 µM

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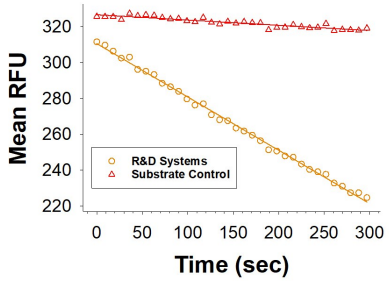
PREPARATION AND STORAGE

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.

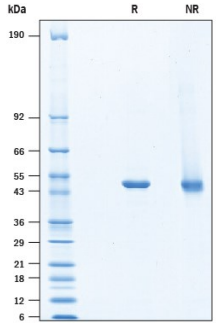
DATA

Enzyme Activity



Recombinant Human ODC1 His-tag Protein (Catalog # 10316-OD) is measured by its ability to convert ornithine to putrescine.

SDS-PAGE



2 µg/lane of Recombinant Human ODC1 His-tag (Catalog # 10316-OD) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 49 kDa under reducing conditions.

BACKGROUND

Ornithine Decarboxylase/ODC1 is a pyridoxal phosphate (PLP)-dependent amino acid decarboxylase enzyme that catalyzes the conversion of ornithine into putrescine in a committed and rate-limiting step for polyamine synthesis. Each 51 kDa enzyme subunit contains a PLP-binding N-terminal domain and a C-terminal domain. Two active sites are formed through dimerization interfaces of the N- and C-terminal domains of opposing subunits (1). Although the enzymatically active form is homodimeric, the dimer is in rapid equilibrium due to weak association of the monomers and allows regulation of ODC1 activity through the binding of antizymes (Az) and antizyme inhibitors (AzIN) as regulatory proteins (2). ODC1 plays a crucial role in regulating polyamine levels (3) associated with cell growth, proliferation, immunity (4), and differentiation. High levels of polyamines and ODC1 are associated with cancer (5). ODC1 is a gene target of the ras and myc oncogenes (6, 7) while also capable of regulating myc expression through putrescine production (8) implicating its role as an oncogene in cancer. Mutation of ODC1 has also been shown to cause developmental delays in Bachman-Bupp syndrome (9). Consequently, ODC1 is a pharmacological target of interest (5) in a growing number of applications such as in Bachman-Bupp Syndrome (9), iron deficiency (10) and multiple cancers including osteosarcoma (11), neuroblastoma (7), breast (12), lung adenocarcinoma (13), hepatocellular carcinoma (14), and endometrial cancer (15).

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