Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

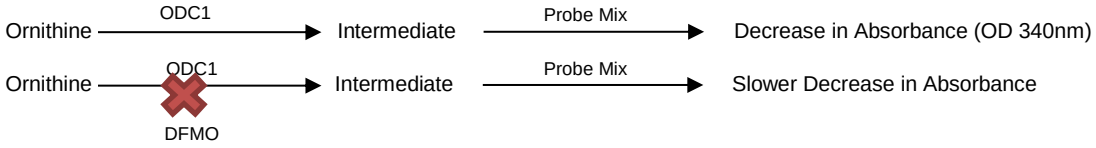
Ornithine Decarboxylase I (ODC1) Inhibitor Screening Kit (Colorimetric)

12/18

(Catalog #K176-100; 100 assays; Store at -20°C)

I. Introduction:

Ornithine decarboxylase (ODC) (EC 3.5.3.1) is a pyridoxal phosphate-dependent enzyme, which cleaves ornithine into putrescine and carbon dioxide. ODC is the initial step in the polyamine synthesis pathway. Putrescine, along with the subsequent polyamine products, spermidine and spermine has been shown to promote cell proliferation and arrest cell apoptosis in cancer. It is also known that proto-oncogenes, such as c-Myc can transcriptionally regulate this protein. Ornithine decarboxylase inhibitors, e.g. efluornithine (2-difluromethylornithine), alexidine and their analogs have been studied as ornithine and employed to irreversibly inhibit ODC and thus abolish cell growth by reducing intracellular polyamine that is required for the aforementioned cellular process. BioVision's Ornithine Decarboxylase I (ODC1) Inhibitor Screening Kit is designed for screening potential human ODC1 inhibitors. DFMO is provided as an inhibitor control. The ODC1 activity is monitored by the decrease in absorbance readings (OD: 340 nm), while potential inhibitors will cause a decrease in the rate of absorbance change. The assay kit is simple, quick and can be used to identify and characterize ODC1 inhibitors in a high-throughput format.



II. Applications:

- Screening for inhibitors of human Ornithine decarboxylase I (ODC1)

III. Kit Contents:

Components	K176-100	Cap Code	Part Number
ODC1 Assay Buffer	25 ml	WM	K176-100-1
ODC1 Substrate	1 vial	White	K176-100-2
ODC1 Converter Mix	1 vial	Purple	K176-100-3
ODC1 Enzyme Mix	1 vial	Green	K176-100-4
ODC1 Cofactor	1 vial	Blue	K176-100-5
Human ODC1	1 vial	Orange	K176-100-6
DFMO (in DMSO)	20 µl	Red	K176-100-7

IV. User Supplied Reagents and Equipment:

- 96-well UV-transparent plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- 1 M DTT
- 50% Glycerol

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Use within two months of opening.

- **ODC1 Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **ODC1 Substrate, ODC1 Converter:** Reconstitute each vial with 220 µl dH_2O. Pipette up and down to dissolve. Store at -20ºC.
- **ODC1 Enzyme Mix:** Reconstitute with 220 µl ODC1 Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.
- **ODC1 Cofactor:** Reconstitute with 1.2 ml dH_2O. Pipette up and down to dissolve. Store at -20ºC. Avoid light. Keep on ice while in use.
- **Human ODC1:** ***Only reconstitute prior to use!*** Mix 5 µl of 1 M DTT solution with 995 µl of 50% glycerol solution (not provided). Add 40 µl of the DTT/50% glycerol solution into the vial. Vortex for 5 seconds. Incubate at 25 ºC for 30 minutes. Completely dissolved Human ODC1 should be a viscous clear yellow solution. Aliquot and store at **-80°C**. Avoid freeze and thaw. Use within two months.
- **DFMO (in DMSO):** Ready to use as supplied. Store at -20°C. Warm to room temperature before use.

VI. ODC1 Inhibitor Screening Assay Protocol:

1. Test compounds, Inhibitor Control, Enzyme Control & Background Control Preparations:

Dissolve candidate inhibitors at 100X highest final test concentration into an appropriate solvent. Dilute to 10X the desired test concentration with ODC1 Assay Buffer. **Sample compound [S]:** Add 10 µl diluted test inhibitor into a 96-well UV-transparent plate (not provided). **For Enzyme Control [EC]:** Add 10 µl of Assay Buffer to a designated well(s). **For Inhibitor control [IC]**: Dilute DFMO by adding 2 µl of the stock solution into 198 µl of ODC1 Assay Buffer. Add 10 µl of the diluted DFMO inhibitor into designated well(s). **For Background Control [BC]**: Add 10 µl of the diluted DFMO in a well designated as Background Control [BC].

Note: If the inhibitor sample or solvent has significant absorbance at 340 nm, add 10 µl diluted test inhibitor in a parallel well designated as **Sample Control [SC]**. Up to 10% DMSO does not affect the reaction. However, it is recommended to study solvent effects by adding the same amount of solvent into parallel well(s) designated as **Solvent Control [SolC]**.

2. **Enzyme Solution Preparation:** Mix enough reagents for the number of assays to be performed. Prepare a 100-fold dilution of the Human ODC1 (e.g. Mix 2 µl of Human ODC1 with 198 µl ODC1 Assay Buffer. *50% glycerol solution is viscous. Handle human ODC1 solution carefully*. For each well, prepare 40 µl ODC1 Enzyme Solution:

ODC1 Assay Buffer	20 µl
Diluted Human ODC1	20 µl

Mix and add 40 µl of the ODC1 enzyme solution to Sample, Inhibitor Control, Enzyme Control and Solvent Control wells ([S], [IC], [EC] and [SolC]). Add 40 µl of Assay Buffer into Background Control and Sample control ([BC] and [SC]) well(s). Mix well, and incubate the plate for 5 min at 37 °C.

Note: Do not store unused diluted DFMO solutions.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix
ODC1 Assay Buffer	34 µl
ODC1 Substrate	2 µl
ODC1 Converter	2 µl
ODC1 Cofactor	10 µl
ODC1 Enzyme Mix	2 µl

Mix and add 50 µl of the Reaction Mix to all wells ([S], [EC], [IC], [SC], [BC], [SolC]). Mix well.

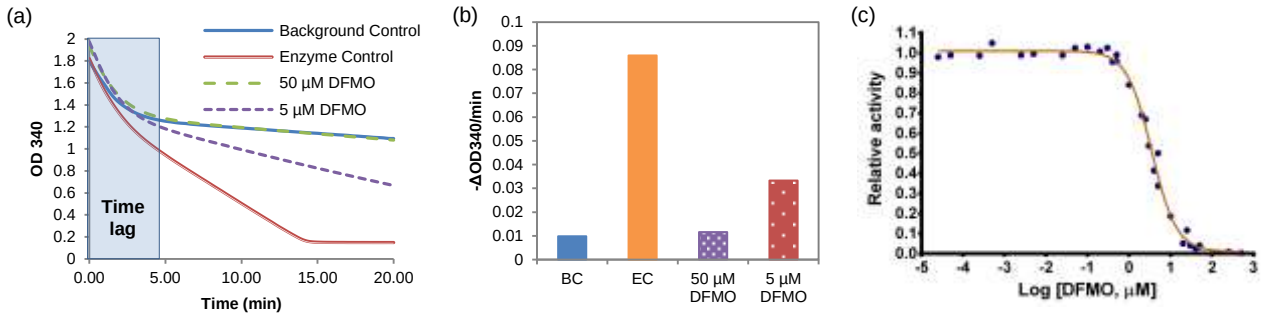
4. **Measurement:** Immediately, measure absorbance (OD: 340 nm) in a microplate reader in kinetic mode at 37°C for 20-30 min, taking measurement readings every 20 seconds.

5. **Calculation:** Take the linear portion of the kinetic curve $\Delta OD = OD_{end} - OD_{initial}$ and divide by reaction time ($\Delta t = t_{end} - t_{initital}$) to get the rate of individual well (note ΔOD rates would be negative). Subtract the Background Control [BC] rate from all readings to obtain activity for each reading (if Sample Control [SC] is higher than Background Control [BC] for a given test compound, subtract its rate from the signal of that particular sample only). Set the activity of Enzyme Control [EC] as 100% (in case Solvent Control is significantly different from EC, replace with [SolC] values in the formulas below). Calculate % Inhibition or % Relative Activity of the test inhibitors as follows:

$$\% \text{ Inhibition} = \frac{\text{Activity of EC} - \text{Activity of S}}{\text{Activtiy of EC}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\text{Activity of S}}{\text{Activity of EC}} \times 100$$

Note: A slow kinetic response in the reactions (Lag phase) may be observed. **Do not use the first 3-5 minutes of the reaction curves for the estimation of of ODC activity**.



Figures: (a) ODC1 was incubated with different amounts of DFMO for 5 minutes at 37 ºC. Kinetic curves were taken for 20 minutes at 37 ºC. (b) Decrease Rates (OD 340/min) of ODC1 incubated with different amounts of DFMO. (c) Inhibition of ODC1 enzyme activity by DL-2-difluoromethylornithine (DFMO). IC_{50} of DL-DFMO: 3.52 ± 0.19 µM. Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Ornithine Decarboxylase, Human Recombinant (P1342)
Ornithine Assay Kit (Kxxx)
Arginase I Inhibitor Screening Assay Kit (K567)
ARG1, Human Recombinant (P1032)
Methionine Assay Kit (Fluorometric) (K442)
Urea Colorimetric Assay Kit (K375)
Total Polyamine Assay Kit (K475)
L-Arginine (Colorimetric) Assay Kit (K749)
Diamine Oxidase Activity Assay Kit (K496)
Arginase 2, Human Recombinant (P1308)
Arginase activity Colorimetric Assay Kit (K755)
Urea Colorimetric Assay Kit II (K376)

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