ab283361 - Ornithine Decarboxylase/ODC Inhibitor Screening Kit (Colorimetric)

For screening for inhibitors of human Ornithine decarboxylase I (ODC1)
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283361

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
ODC1 Assay Buffer	25 mL	-20°C
ODC1 Substrate	1 vial	-20°C
ODC1 Converter Mix	1 vial	-20°C
ODC1 Enzyme Mix	1 vial	-20°C
ODC1 Cofactor	1 vial	-20°C
Human ODC1	1 vial	-20°C
DFMO (in DMSO)	20 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

– Before using the kit, spin the tubes prior to opening.

ODC1 Assay Buffer: Warm to room temperature before use. Store at 4°C or -20°C.

ODC1 Substrate, ODC1 Converter: Reconstitute each vial with 220 µl dH_2O. Pipette up and down to dissolve. Store at -20°C.

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 -20°C. 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 °C 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.

Screening Protocol

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

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

ΔNote: *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].*

Enzyme Solution Preparation:

1. Mix enough reagents for the number of assays to be performed.
2. 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.
3. For each well, prepare 40 µl ODC1 Enzyme Solution:

Item	Amount
ODC1 Assay Buffer	20 µl
Diluted Human ODC1	20 µl

4. 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]).
5. 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.

ΔNote: *Do not store unused diluted DFMO solutions.*

Reaction Mix:

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

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

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

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.

Calculation:

1. Take the linear portion of the kinetic curve ΔOD= 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).
2. 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).
3. 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).
4. Calculate % Inhibition or % Relative Activity of the test inhibitors as follows:

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

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

ΔNote: *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 ODC activity.*

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)