

ab283362 - Glutamate Dehydrogenase Inhibitor Screening Kit (Fluorometric)

For the screening of potential inhibitors of Glutamate Dehydrogenase (GDH)

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab283362>

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
GDH Assay Buffer	25 mL	-20°C
GDH Substrate	200 µl	-20°C
GDH Developer	1 vial	-20°C
GDH Probe	400 µl	-20°C
GDH Enzyme	1 vial	-20°C
GDH Inhibitor	1 vial	-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 white plate with flat bottom
- Multi-well spectrophotometer
- Distilled Water
- Glycerol

Reagent Preparation

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

GDH Assay Buffer: Warm to room temperature before use.

GDH Substrate and Probe: Thaw GDH substrate and Probe at room temperature before use.

Aliquot and store at -20°C in the dark.

GDH Developer: Reconstitute with 220 µl GDH Assay Buffer. Aliquot and store at -20°C.

GDH Enzyme: Reconstitute with 220 µl of 50% glycerol (50% glycerol made in water). Mix well.

Aliquot and store at -20°C. Lyophilized enzyme is stable up to 12 months at -20°C

GDH Inhibitor: Reconstitute with 44 µl water. Aliquot and store at -20°C.

ΔNote: Keep GDH Enzyme Mix and GDH Developer on ice while performing the assay

Screening Protocol

GDH Inhibitor Screening Assay Protocol:

Test Inhibitor Preparation: Dissolve the Test Inhibitor in appropriate solvent. Prepare at concentrations so that the volume of the Test Inhibitor solution added to a well is no more than 5 µl in the final 100 µl reaction volume per well. Add Test Inhibitor to each test well. Bring the volume up to 50 µl in each well by adding GDH Assay Buffer.

For Solvent Control: Add Solvent used to prepare Test Inhibitor solution at its final concentration in inhibitor wells to solvent control wells. Bring the volume up to 50 µl in each well by adding GDH Assay Buffer.

For Inhibitor Control: Add 4 µl of GDH Inhibitor and bring up the volume to 50 µl in each well by adding GDH Assay Buffer.

For Enzyme Control: Add 50 µl of GDH Assay Buffer in each well.

Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Mix containing:

Item	Reaction Mix
GDH Assay Buffer	18 µl
GDH Enzyme	2 µl

Add Reaction Mix to Test Inhibitor, Solvent Control, Inhibitor Control and Enzyme Control wells. Incubate at RT for 10 min.

Substrate Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare 30 µl Mix containing:

Item	Reaction Mix
GDH Assay Buffer	24 µl
GDH Substrate	2 µl
GDH Developer	2 µl
GDH Probe	2 µl

Mix well and add 30 µl of the Substrate Mix to all wells including Test Inhibitor, Solvent Control, Inhibitor Control and Enzyme Control

Measurement:

Read fluorescence in a kinetic mode at Ex/Em = 535/587 nm at 30 second intervals for 10-20 min at 37°C

Calculation:

Obtain Δ RFU for all Test Inhibitors, Enzyme Control, Solvent Control and Inhibitor Control by subtracting RFU at time t₁ from RFU at time t₂, such that t₂ and t₁ is within a linear range of the assay.

Calculate slope for all Samples, including Enzyme Control by dividing ΔRFU by time Δt (t₂ - t₁). If Solvent Control slope is significantly different from Enzyme Control slope, use its values instead of Enzyme Control in the calculations shown below.

$$\% \text{ Inhibition} = \frac{[\text{slope of (enzyme control)} - \text{slope of (test compound)}]}{\text{Activtiy of slope of (enzyme control)}} \times 100$$

$$\% \text{ Relative activity} = \frac{[\text{slope (test compound)}]}{\text{slope (enzyme control)}} \times 100$$

Technical Support

Copyright © 2023 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)