3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Aldehyde Dehydrogenase Inhibitor Screening Kit

Catalog Number **MAK327**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Aldehyde Dehydrogenases (ALDHs) are a superfamily of oxidoreductases which catalyze the conversion of aldehydes to carboxylic acids. ALDH is crucial in the metabolism of alcohol as alcohol dehydrogenase breaks down ethanol to acetaldehyde. Acetaldehyde, which is toxic to the body, is in turn broken down by ALDH to acetic acid. Imbalances of aldehyde dehydrogenase have been linked to both alcoholism and alcohol sensitivity in people. Inhibitors of the enzyme have been used in cases to treat alcoholism in patients. Cancer stem cell populations also display a heightened activity of ALDH, making ALDH inhibition a promising anticancer therapy approach.

The Aldehyde Dehydrogenase Inhibitor Screening Kit is based on the enzymatic conversion of acetaldehyde and NAD to acetic acid and NADH by ALDH. This NADH in turn reduces a formazan reagent into a colored product the absorbance of which, measured at 565 nm, is proportional to the enzymatic activity in the reaction. The percent inhibition of a test compound can be determined by comparing the activity of ALDH treated with the test compound to the activity of untreated ALDH.

This kit can be readily automated on HTS liquid handling systems and is suitable for inhibitor screening and evaluation of ALDH inhibitors.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.
Note: Neither the enzyme ALDH nor a control inhibitor are included in the kit.

Assay Buffer Catalog Number MAK327A	12 mL
NAD/MTT Catalog Number MAK327B	1 mL
Diaphorase Catalog Number MAK327C	120 µL
4× Substrate (400 mM) Catalog Number MAK327D	50 µL

Reagents and Equipment Required but Not Provided.

- Purified Aldehyde Dehydrogenase (Catalog Number A6338)
- If desired, a control Aldehyde Dehydrogenase Inhibitor: Disulfiram (Catalog Number 86720)
- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well flat bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reagent Preparation

Prior to assay, equilibrate all components to room temperature. Keep 4× Substrate and Diaphorase on ice. Pre-warm Assay Buffer to 25 °C.

Sample Preparation - The following protocol is optimized for ALDH from baker's yeast. If another species is being analyzed, it is recommended to experimentally determine the K_M value and then adjust the volume of substrate in the Reaction Mix so that the final concentration of the substrate in the 100 µL reaction is near the K_M.

Enzyme Preparation - Dilute the purified ALDH to 22 U/mL using Assay Buffer.

Inhibitor Solution - Dissolve the test compounds (i.e., inhibitors) in solvent of choice. It is prudent to first test the tolerance of the solvent by the enzyme being tested. DMSO at concentrations of 5% (v/v) or less in the final 100 µL of reaction volume will not interfere with the reaction (the 5 µL of test compounds may be in 100% DMSO).

1× Substrate - Prepare enough 1× Substrate by diluting 4× Substrate 4-fold in ultrapure water. Each well will need 1 µL of 1× Substrate.

Reaction Mixes - Prepare the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for all wells.

Table 1.
Reaction Mixes

Reagent	Sample Volume	Blank Volume
Assay Buffer	45 µL	45 µL
NAD/MTT	8 µL	8 µL
Diaphorase	1 µL	1 µL
1× Substrate	1 µL	–

Note: The Reaction Mix should be prepared fresh and used within two hours.

Storage/Stability

The kit is shipped on dry ice. Store all reagents at –20 °C.

Procedure

Note: This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Reaction Mix should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended. Neither the enzyme ALDH nor a control inhibitor is included in the kit.

ALDH Reaction Assay

1. Transfer 45 µL of ALDH Enzyme Preparation into separate wells.
2. Reserve two ALDH wells for the Blank (No Substrate) and the Control (No Inhibitor).
3. To the Control and Blank wells, add 5 µL of the solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 100% DMSO, add 5 µL of 100% DMSO to these wells.
4. To the remainder of the wells containing ALDH, add 5 µL of the test compounds.
5. Add 50 µL of the Blank Reaction Mix to the Blank well. Add 50 µL of Sample Reaction Mix to the remaining wells. Tap plate to mix briefly and thoroughly.
6. Incubate the plate for 30 minutes at room temperature.
7. Measure the absorbance at 565 nm (A_{565}).

Results

ALDH inhibition for a test compound is calculated as follows:

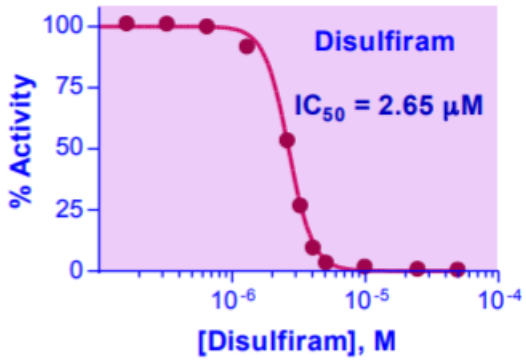
$$\% \text{ Inhibition} = (1 - A_{565\ \underline{\text{Test Cpd}}}/A_{565\ \text{No Inhibitor}}) \times 100\%$$

where:

$A_{565\ \text{Test Cpd}}$ = the A_{565} value of a test compound well minus the A_{565} value of the Blank well (No Substrate) at 30 minutes

$A_{565\ \text{No Inhibitor}}$ = the A_{565} value of a Control well (No Inhibitor) minus the A_{565} value of the Blank well (No Substrate) at 30 minutes

Figure 1.
Disulfiram Titrations



ALDH from baker's yeast was incubated with various concentrations of Disulfiram. Each concentration of inhibitor contained 10% (v/v) DMSO [final 0.5% (v/v) in 100 µL reaction).

References

1. Raha, D. et al., The Cancer Stem Cell Marker Aldehyde Dehydrogenase is Required to Maintain a Drug-Tolerant Tumor Cell Subpopulation. Cancer Res., **74(13)**, 3579-90 (2014).
2. Kang, J.H. et al., Aldehyde Dehydrogenase inhibition combined with phenformin treatment reversed NSCLC through ATP Depletion. Oncotarget, **7**, 49397-49410 (2016).
3. Koppaka, V. et al., Aldehyde Dehydrogenase Inhibitors: a Comprehensive Review of the Pharmacology, Mechanism of Action, Substrate Specificity, and Clinical Application. Pharmacol. Rev., **64(3)**, 520-539 (2012).

HM,MAM 11/18-1