QuantiChrom™ Aldehyde Dehydrogenase Inhibitor Screening Kit (EIAL-100)

Quantitative Determination of Aldehyde Dehydrogenase Inhibitor Activity at 565 nm

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 anti-cancer therapy approach.

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

KEY FEATURES

Rapid and reliable. Can be completed in under an hour.

Homogenous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS: can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

HTS for inhibitor screening and evaluation of ALDH inhibitors.

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer:	12 mL	**Diaphorase**:	120 µL
NAD/MTT:	1 mL	**4×Substrate (400 mM)**:	50 µL

Bulk Reagents: Custom sizes available upon request.

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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

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

Sample Preparation: The following protocol is optimized for ALDH from baker's yeast. If another species is being analyzed, we recommend that you experimentally determine the K_m and then adjust the volume of substrate in the Working reagent so that the final concentration of the substrate in the 100 µL reaction is near the K_m.

Dilute purified ALDH to 22 U/mL using assay buffer. 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 of choice. DMSO at concentrations of 5 v/v% or less in the final 100 µL reaction volume will not interfere with the reaction (the 5 µL of test compounds may be in 100% DMSO).

ALDH Reaction Preparation:

1. Transfer 45 µL of 22 U/mL ALDH into separate wells.
2. Reserve two ALDH wells for the Blank (no substrate) and Control (no inhibitor).
3. To the Control and Blank wells, add 5 µL of solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 100% DMSO, add 5 µL 100% DMSO to these wells.
4. To the remainder of the wells containing ALDH, add 5 µL of the test compounds.
5. Prepare enough 1× Substrate by diluting 4× Substrate 4-fold in dH_2O. (Each well will need 1 µL of 1× Substrate)
6. Prepare sufficient Reaction Mix (RM) by mixing for each well (except Blank well), 45 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Diaphorase, and 1 µL 1× Substrate.

   Prepare Blank Reaction Mix (BRM) by mixing for each blank well, 45 µL Assay Buffer, 8 µL NAD/MTT, and 1 µL Diaphorase (*i.e. No 1× Substrate*).

   Add 50 µL BRM to the Blank well. Add 50 µL RM to the remaining wells. Tap plate to mix briefly and thoroughly.
7. Incubate the plate for 30 minutes at room temperature and read optical density at 565 nm

CALCULATION

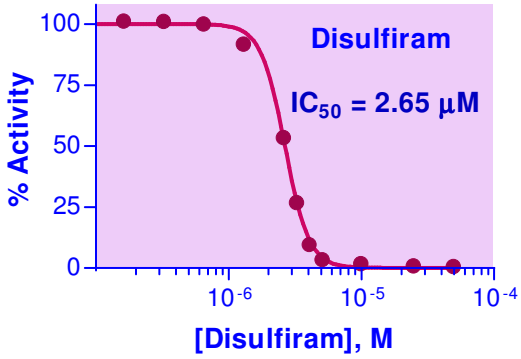
ALDH inhibition for a test compound is calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta OD_{\text{Test Cpd}}}{\Delta OD_{\text{No Inhibitor}}}\right) \times 100\%$$

Where $\Delta OD_{\text{Test Cpd}}$ is the OD_{565nm} value of a test compound minus the OD_{565nm} value of the Blank well at 30 min and $\Delta OD_{\text{No Inhibitor}}$ is the OD_{565nm} value of the Control minus the OD_{565nm} value of the Blank well at 30 min.

MATERIALS REQUIRED, BUT NOT PROVIDED

Purified ALDH (e.g. Sigma Aldrich cat# A6338) and if desired a control ALDH inhibitor (e.g. Disulfiram, Sigma Aldrich Cat# PHR1690). Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



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).

LITERATURE

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