Aldehyde Dehydrogenase Inhibitor Screening Kit

Cat. No. KITG-154 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Quantitative determination of aldehyde dehydrogenase inhibitor activity at 565 nm.
Description	The 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. Scientific Background 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. Kit Components Assay Buffer: 12 mL Diaphorase: 120 µL NAD/MTT: 1 mL 4× Substrate (400 mM): 50 µL Bulk Reagents: custom sizes available upon request. Materials Required but Not Supplied Purified ALDH and, if desired, a control ALDH inhibitor. Pipetting devices and accessories (e.g., multi-channel pipettor), clear flat-bottom 96-well plates, and a plate reader.
Applications	HTS for inhibitor screening and evaluation of ALDH inhibitors.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Notes	The following protocol is optimized for ALDH from baker's yeast. If another species is being analyzed, we recommend that you experimentally determine the Km 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 Km. Note: neither the enzyme ALDH nor a control inhibitor is included in the kit. Reagents are for research use only. Please refer to the Material Safety Data Sheet for detailed information.
Storage	Store all reagents at -20 °C. Shelf life of six months after receipt. Keep 4× Substrate and Diaphorase on ice.
Shipping	The kit is shipped on ice.
Reaction conditions	This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of the Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
Usage	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 Km 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 Km. 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). Assay Protocol 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 the solvent that the test

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	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 the Blank well), 45 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Diaphorase, and 1 µL 1× Substrate. 7. 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). 8. Add 50 µL BRM to the Blank well. Add 50 µL RM to the remaining wells. Tap the plate to mix briefly and thoroughly. 9. Read the optical density at 565 nm at 30 min. Assay Time Can be completed in under an hour; absorbance at 565 nm is recorded at 30 min. Analysis % Inhibition = $(1 - \Delta OD_{(Test\ Cpd)} / \Delta OD_{(No\ Inhibitor)}) \times 100$. Where $\Delta OD_{(Test\ Cpd)}$ is the OD_{565} nm value of a test compound minus the OD_{565} nm value of the Blank well at 30 min, and $\Delta OD_{(No\ Inhibitor)}$ is the OD_{565} nm value of the Control minus the OD_{565} nm value of the Blank well at 30 min.
Inhibitors	Test compounds (i.e., inhibitors) dissolved in solvent of choice.

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