Aldehyde Dehydrogenase Inhibitor Screening Kit

Aldehyde Dehydrogenase Inhibitor Screening Kit is a screening kit for inhibitor screening and evaluation of aldehyde dehydrogenase inhibitors.

Catalog number: ARG82134

Package: 100 tests

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Alcohol dehydrogenases (ADH) (EC 1.1.1.1) are a group of dehydrogenase enzymes that occur in many organisms and facilitate the interconversion between alcohols and aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. In humans and many other animals, they serve to break down alcohols that otherwise are toxic, and they also participate in generation of useful aldehyde, ketone, or alcohol groups during biosynthesis of various metabolites. In yeast, plants, and many bacteria, some alcohol dehydrogenases catalyze the opposite reaction as part of fermentation to ensure a constant supply of NAD^+. [Provide by Wikipedia: Alcohol dehydrogenase]

PRINCIPLE OF THE ASSAY

This Aldehyde Dehydrogenase Inhibitor Screening Kit is a simple colorimetric assay that measures the amount of ADH Inhibitor in samples. This assay 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 at O.D. 565 nm. The optical density 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.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at-20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	12 mL	-20°C
NAD / MTT	1 mL	-20°C
Diaphorase	120 µL	-20°C
4X Substrate (400 mM)	50 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Centrifuge
- Clear flat-bottom 96 well microplate
- Purified ALDH (E.g., Sigma Aldrich cat# A6338)
- ALDH inhibitor (E.g., Sigma Aldrich cat# PHR1690)
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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 of reaction is near the Km.

Working ALDH Solution: Dilute purified ALDH to 22 U/mL using Assay Buffer. Dissolve the test compounds (E.g., 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).

REAGENT PREPARATION

- 1X Substrate: diluting 4X Substrate 4-fold in distilled water. (Each well need 1 µL of 1X Substrate)
- Reaction Mix: for each 96 well assay, mix 1 µL of 1X Substrate, 8 µL of MTT / NAD Solution, 1 µL of Diaphorase and 45 µL of Assay Buffer. Fresh preparation before assay.
- Blank Reaction Mix: for each 96 well assay, mix 8 µL of MTT / NAD Solution, 1 µL of Diaphorase and 45 µL of Assay Buffer. Fresh preparation before assay.

ASSAY PROCEDURE

Equilibrate reagents to desired reaction temperature (E.g., 25°C or 37°C). Briefly centrifuge tubes before use.

	Sample	Blank	Control
Working ALDH Solution (22 U/mL)	45 µL	45 µL	45 µL
Solvent (test compounds are dissolved in)		5 µL	5 µL
Test compounds	5 µL		
Blank Reaction Mix		50 µL	
Reaction Mix	50 µL		50 µL
Tap microplate to mix briefly and thoroughly. Incubate for 30 minutes at room temperature. And read the absorbance at O.D. 565 nm.			

CALCULATION OF RESULTS

1. ALDH inhibition for a test compound is calculated as follows:

   % Inhibition = $(1 - \Delta OD_{Test} / \Delta OD_{No\ inhibitor}) \times 100\%$

   Note:

   - ΔOD_{Test}: the $OD_{565\ nm}$ value of a test compound minus $OD_{565\ nm}$ value of the Blank well at 30 minutes.
   - $\Delta OD_{No\ inhibitor}$: the $OD_{565\ nm}$ value of the Control minus the $OD_{565\ nm}$ value of the Blank well at 30 minutes.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Aldehyde Dehydrogenase Inhibitor Screening Kit. One should use the data below for reference only. This data should not be used to interpret actual results.