EnzyChrom™ Aldehyde Dehydrogenase Assay Kit (EADH-100)

Quantitative Colorimetric Assay for Aldehyde Dehydrogenase Activity

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 ALDHs have been linked to both alcoholism and alcohol sensitivity in people.

BioAssay Systems' EADH-100 Kit is based on the enzymatic conversion of acetaldehyde to acetic acid and NADH by ALDH. The formed NADH is converted into a colored product, the absorbance of which, measured at 565 nm, is proportional to the enzyme activity in the reaction.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.2 - 25 U/L ALDH in a 96-well plate assay.

Convenient. The assay involves addition of a single working reagent and can be completed in under an hour.

Robust and amenable to HTS. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

For quantitative determination of aldehyde dehydrogenase activity in biological samples (e.g serum, cell lysate, etc).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	12 mL	**Diaphorase:**	120 µL
NAD/MTT:	1 mL	**4x Substrate (400mM):**	50 µL
Calibrator	1.5 mL		

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent (*WR*) to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. The assay can be run at room temperature.

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The *WR* should be prepared fresh for each assay run.

Enzyme Preparation: Enzyme should be prepared in an enzyme buffer, e.g. 100 mM Tris-HCl, pH 8, 100 mM KCl, 0.09% BSA. The following protocol is optimized for ALDH from Sigma Aldrich (Cat # A6338). If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

Sample Preparation:

Serum samples should be diluted 3-fold or higher prior to the assay.

Cell lystate can be assayed directly.

Reaction Preparation:

1. Transfer 100 µL H2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.

2. Transfer 50 µL of each sample to separate wells of the plate.

3. Prepare enough *WR* for all wells by mixing for each well 45 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Diaphorase, and 0.25 µL 4x Substrate.

4. Add 50 µL *WR* to all Sample wells. Briefly tap plate to mix and read optical density (OD_{565}) at 0 min and at 30 min.

CALCULATION

ALDH activity can be calculated as follows:

$$\text{ALDH Activity} = \frac{(OD_{30} - OD_0)}{\varepsilon_{mtt} \times l} \times \frac{\text{Reaction Vol (µL)}}{t\,(\text{min}) \times \text{Enzyme Vol (µL)}} \times n \text{ (U/L)}$$

$$= 3.64 \times \frac{(OD_{30} - OD_0)}{(OD_{Cal} - OD_{H2O})} \times n \text{ (U/L)}$$

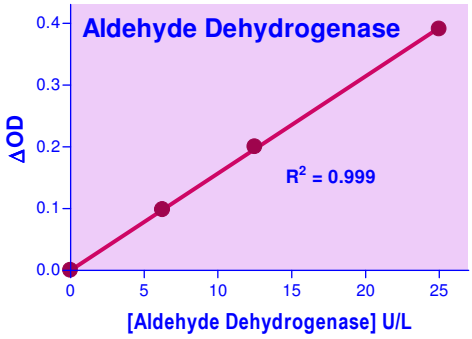
Where OD_{30} and OD_0 are the measured OD_{565} values of the sample at 30 min and 0 min. ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength that is calculated from the calibrator. OD_{Cal} and OD_{H2O} are the OD_{565} values of the calibrator and water. Reaction Vol and Sample Vol are 100 µL and 50 µL, respectively. t is the reaction time (30 min.) and n is the sample dilution factor.

Unit definition: 1 Unit (U) of ALDH will catalyze the conversion of 1 µmole of the Substrate per min at room temperature and pH 8.

Note: If sample ALDH activity exceeds 25 U/L, dilute samples in enzyme buffer and repeat the assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear, flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and a plate reader.



96-well Colorimetric Aldehyde Dehydrogenase Assay

Examples: Human serum and cell lysate from HEPG2 liver cells were assayed for ALDH activity. The baseline levels were 2.0 ± 0.3 U/L and 8.9 ± 0.2 U/L, respectively.

LITERATURE

1. Jean, E. et al. (2011). Aldehyde dehydrogenase activity promotes survival of human muscle precursor cells. Journal of cellular and molecular medicine. 15, 119-33.

2. Klyosov, A. (1996). Kinetics and Specificity of Human Liver Aldehyde Dehydrogenases toward Aliphatic, Aromatic, and Fused Polycyclic Aldehydes. Biochemistry. 35 (14), 4457-4467.

3. Shortall, K, et al. (2021). Insights into Aldehyde Dehydrogenase Enzymes: A Structural Perspective. Front. Mol. Biosci., 8 (1).