Aldehyde Dehydrogenase Assay Kit (BA0234)

Product Datasheet | SKU BA0234

Description

A quantitative colorimetric assay for aldehyde dehydrogenase activity based on the enzymatic conversion of acetaldehyde to acetic acid and NADH by ALDH. The formed NADH is converted into a coloured product measured at 565 nm, proportional to enzyme activity, with a linear detection range of 0.2 - 25 U/L ALDH.

Specifications

Detection Method	Colorimetric determination at OD565 nm
Detection Range	0.2 - 25 U/L ALDH in a 96-well plate assay
Sample Type	Biological samples (e.g. serum, cell lysate)
Species Reactivity	All
Assay Time	30 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped at room temperature. Store all components at -20°C upon receipt.
Shelf Life	6 months after receipt.
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	12 mL	-20°C
Diaphorase	120 µL	-20°C
NAD/MTT	1 mL	-20°C
4x Substrate (400 mM)	50 µL	-20°C
Calibrator	1.5 mL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0234	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Equilibrate all components to room temperature and briefly centrifuge tubes before opening. The Working Reagent (WR) should be prepared fresh for each assay run.
2. Prepare the enzyme in an enzyme buffer, e.g. 100 mM Tris-HCl, pH 8, 100 mM KCl, 0.09% BSA, when required.
3. Sample preparation: serum samples should be diluted 3-fold or higher prior to the assay; cell lysate can be assayed directly.
4. Transfer 100 µL H2O (OD_H2O) and 100 µL Calibrator (OD_CAL) solution into wells of a clear flat-bottom 96-well plate.
5. Transfer 50 µL of each sample to separate wells of the plate.
6. Prepare enough WR for all wells by mixing, per well, 45 µL Assay Buffer, 8 µL NAD/MTT, 1 µL Diaphorase and 0.25 µL 4x Substrate.

7. Add 50 µL WR to all Sample wells. Briefly tap plate to mix and read optical density (OD565) at 0 min and at 30 min.

Calculation

ALDH Activity = [(OD30 – OD0) / (ε_mtt × l)] × [Reaction Vol / (t × Enzyme Vol)] × n = 3.64 × [(OD30 – OD0) / (OD_Cal – OD_H2O)] × [Reaction Vol / (t × Enzyme Vol)] × n (U/L), where OD30 and OD0 are the OD565 values at 30 and 0 min, ε_mtt is the molar absorption coefficient of reduced MTT, l is the light pathlength from the calibrator, Reaction Vol is 100 µL, Enzyme Vol is 50 µL, t is 30 min and n is the sample dilution factor. Unit definition: 1 U of ALDH catalyses the conversion of 1 µmole of Substrate per min at room temperature and pH 8. If sample activity exceeds 25 U/L, dilute in enzyme buffer and repeat.

Notes

- This assay is based on a kinetic reaction; addition of the Working Reagent to samples should be quick and mixing brief but thorough.
- The assay can be run at room temperature.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.