Aldehyde Dehydrogenase Inhibitor Screening Kit (BA0249)

Product Datasheet | SKU BA0249

Description

A quantitative colorimetric inhibitor screening assay for aldehyde dehydrogenase (ALDH). ALDH converts acetaldehyde to acetic acid and NADH, which reduces a formazan reagent to a coloured product measured at 565 nm; percent inhibition is derived by comparison with untreated ALDH.

Specifications

Detection Method	Colorimetric (OD565nm)
Sample Type	Purified aldehyde dehydrogenase and test compounds
Species Reactivity	All
Assay Time	Under one hour
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months after receipt
Shipping	Gel Pack

Kit Components

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

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0249	100 Assays

Assay Procedure

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

1. This assay is based on an enzyme-catalysed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing brief but thorough; a multi-channel pipettor is recommended. Note: neither the ALDH enzyme nor a control inhibitor is included in the kit.
2. Reagent preparation: equilibrate all components to room temperature. Keep 4Substrate and Diaphorase on ice. Pre-warm Assay Buffer to 25°C. Prepare the Reaction Mix fresh and use within two hours.
3. Sample preparation (optimised for ALDH from baker's yeast): for other species, determine the Km and adjust the substrate volume so the final concentration in the 100 µL reaction is near the Km. Dilute purified ALDH to 22 U/mL using assay buffer. Dissolve test compounds in a solvent of choice (DMSO at 5 v/v% or less in the final reaction will not interfere).
4. Transfer 45 µL of 22 U/mL ALDH into separate wells. Reserve two ALDH wells for the Blank (no substrate) and Control (no inhibitor).
5. To the Control and Blank wells add 5 µL of the solvent used for the test compounds. To the remaining ALDH wells add 5 µL of the test compounds.
6. Prepare enough 1 Substrate by diluting 4 Substrate 4-fold in dH2O (each well needs 1 µL). Prepare sufficient Reaction Mix (RM) by mixing, per well (except blank): 45 µL Assay Buffer, 8 µL NAD/MTT, 1 µL

Diaphorase and 1 µL 1 Substrate. Prepare Blank Reaction Mix (BRM) per blank well: 45 µL Assay Buffer, 8 µL NAD/MTT and 1 µL Diaphorase (no 1 Substrate). Add 50 µL BRM to the Blank well and 50 µL RM to the remaining wells. Tap 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: % Inhibition = (1 – ΔOD_TestCpd / ΔOD_NoInhibitor) × 100%, where ΔOD_TestCpd is the OD565nm value of a test compound minus the OD565nm value of the Blank well at 30 min, and ΔOD_NoInhibitor is the OD565nm value of the Control minus the OD565nm value of the Blank well at 30 min.

Notes

- Bulk reagents and custom sizes are available upon request.
- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.