3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

High Sensitivity Aldehyde Dehydrogenase Activity Fluorometric Assay Kit

Catalog Number **MAK282**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

NAD-dependent Aldehyde Dehydrogenase (ALDH) plays a vital role in cellular detoxification. ALDH oxidizes various aldehydes and generates the corresponding carboxyolic acid. NAD-dependent Aldehyde Dehydrogenase have been found in every cellular compartment. Based on its structure and function, ALDH comprises 3 major classes in mammals: Class 1 and Class 3 (the tumor form) are located in the cytosol and include both constitutive and induced forms; Class 2 is located in the mitochondria and only exists as the constitutive form.

In humans, the ALDH superfamily consists of 19 genes. The mutation of ALDH genes (loss of function) causes human diseases such as Type II hyperprolinemia, pyridoxine-dependent seizure, and hyperammonemia. Recent studies show increased ALDH activity leads to several types of malignancies, serves as a cancer stem cell marker, and correlates with poor prognosis. Therefore, the early detection of ALDH activity levels can be prognostic and guide the therapeutic strategies.

This High Sensitivity Aldehyde Dehydrogenase Activity Assay Kit is a robust tool to quantify ALDH enzymatic activity. In this assay, acetaldehyde is oxidized by ALDH to form NADH, which couples with the High Sensitivity Probe to generate a potent fluorescence (λ_{ex} = 535 nm/λ_{em} = 587 nm). The ALDH fluorometric assay kit is 10 times more sensitive than the ALDH colorimetric assay and can detect <0.05 mU of ALDH activity in a variety of samples, based on the following unit definition:

Unit Definition: One unit is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 8 at room temperature.

Components

The kit is sufficient for 100 assays in 96 well plates.

ALDH Assay Buffer Catalog Number MAK282A	25 mL
Acetaldehyde Catalog Number MAK282B	0.5 mL
High Sensitivity Probe (in DMSO) Catalog Number MAK282C	0.4 mL
ALDH Substrate Mix Catalog Number MAK282D	1 vial
ALDH Positive Control Catalog Number MAK282E	1 vial
NADH Standard (0.5 μmole) Catalog Number MAK282F	1 vial

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates for this assay.
- Fluorescence multiwell plate reader
- Glycerol (Catalog Number G6279 or equivalent)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge all small vials prior to opening. Use ultrapure water for the preparation of reagents.

ALDH Assay Buffer and Acetaldehyde – Store at –20 °C. Let ALDH Assay Buffer warm to room temperature before use.

Aldehyde Dehydrogenase Substrate Mix – Reconstitute with 220 µL of ALDH Assay Buffer. Pipette up and down to completely dissolve. Store at –20 °C. Use within two months.

ALDH Positive Control – Reconstitute with 220 µL of Assay Buffer containing 20% glycerol (not included). Pipette up and down to completely dissolve, aliquot, and store at –20 °C. Avoid repeated freeze/thaw cycles.

NADH Standard – Reconstitute with 500 µL of water to generate 1 mM NADH. Aliquot and store at –20 °C. Avoid repeated freeze/thaw cycles.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended. Let ALDH Assay Buffer warm to room temperature before use. Briefly centrifuge all small vials prior to opening.

Procedure
All samples and standards should be run in duplicate.

NADH Standard Curve
Dilute the NADH Standard to 0.05 mM by adding 10 µL of the 1 mM NADH Standard to 190 µL of Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 µL into a 96 well plate in duplicate to generate 0, 100, 200, 300, 400, 500 pmole/well standards, adjust volume to 50 µL/well with ALDH Assay Buffer.

For samples having very low ALDH activity, Add 0, 1, 2, 3, 4, 5 µL into a 96 well plate in duplicate to generate 0, 50, 100, 150, 200, 250 pmole/well standards, adjust volume to 50 µL/well with ALDH Assay Buffer.

Sample Preparation
Liquid samples can be measured directly. Tissue (50 mg) or cells (1×10^6) should be rapidly homogenized with ~200 µL of ice cold ALDH Assay Buffer for 10 minutes on ice, then spun down at 12000 rpm for 5 minutes to remove nuclei and insoluble material. Add 1–50 µL of the collected supernatant into a 96 well plate and adjust the final volume to 50 µL with ALDH Assay Buffer.

Notes
For unknown samples, testing several concentrations of the samples to ensure the readings are within the Standard Curve range is suggested. NADH in samples will generate a background reading. Background readings can be corrected by omitting the Acetaldehyde in the Reaction Mix as a background control. For the optional Positive Control, dilute the reconstituted Positive Control 10-fold in Assay Buffer then use 5–10 µL and adjust the final well volume to 50 µL with Assay Buffer

Reaction Mix
Mix enough of the appropriate Reaction Mix for the number of samples and standards to be performed: For each well, prepare 50 µL of the appropriate Reaction Mix, see Table 1.

Table 1.
Preparation of Reaction Mixes

Reagent	ALDH Measurement	Background Control
ALDH Assay Buffer	39 µL	44 µL
High Sensitivity Probe**	2 µL	2 µL
Substrate Mix	2 µL	2 µL
Acetaldehyde	5 µL	–

**Note: For NADH standards or samples, which will generate less than 250 pmole of NADH, reduce the probe volume to 1 µL per well to reduce reagent background and increase the assay buffer accordingly.

Add 50 µL of the appropriate Reaction Mix to each well containing the NADH Standard, test samples, and background controls, mix well.

Measurement
Incubate at room temperature for 5 minutes protected from light. Measure the initial RFU of samples and sample backgrounds at λ_{ex} = 535 nm/λ_{em} = 587 nm (RFU_1 and RFU_{1B}), then measure the RFU at λ_{ex} = 535 nm/λ_{em} = 587 nm again after 20–60 minutes (RFU_2 & RFU_{2B}) depending on the ALDH activity in the samples. NADH standards can be measured at the end point. Measuring the samples in a kinetic mode (every 2–3 minutes) and picking a linear range within the NADH Standard Curve is suggested.

Results

Calculation

Subtract the 0 Standard reading from all Standard readings and plot the Standard Curve. Compare the ΔRFU λ_{ex} = 535 nm/λ_{em} = 587 nm of the sample [($RFU_2 - RFU_{2B}$) – ($RFU_1 - RFU_{1B}$)] to the Standard Curve to obtain the amount (B, in nmole) of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

ALDH activity = $B/(\Delta T \times V) \times$ Sample Dilution Factor (pmole/min/mL = μU/mL)

B = amount of NADH generated in sample (pmole)
ΔT = the reaction time (minutes)
V = a sample volume (mL)

Unit Definition: One unit is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 8 at room temperature.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	It is recommended to use black plates for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

SJ,MAM 04/16-1