

Aldehyde Dehydrogenase Activity Colorimetric Assay Kit

(Catalog #BN00950; 100 assays; Store Kit at -20°C)

I. Introduction:

The NAD-dependent Aldehyde Dehydrogenase (ALDH) plays a vital role in cellular detoxification. It oxidizes various aldehydes and generates the corresponding carboxylic acid. ALDH 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 that 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. The Assay Genie Aldehyde Dehydrogenase (ALDH) Activity Assay Kit is a simple, fast and reliable method to quantify the ALDH enzymatic activity. In this assay, acetaldehyde is oxidized by ALDH generating NADH which then reduces a colorless probe to a colored product with strong absorbance at 450 nm. The assay can detect < 0.5 mU of ALDH activity (based on our unit definition) in a variety of samples.

II. Kit Contents:

Components	BN00950	Cap Code	Part Number
ALDH Assay Buffer	25 ml	WM	BN00950-1
Acetaldehyde	0.5 ml	Purple	BN00950-2
ALDH Substrate Mix (Lyophilized)	1 vial	Red	BN00950-3
ALDH Positive Control (Lyophilized)	1 vial	Green	BN00950-4
NADH Standard (0.5 μmol, Lyophilized)	1 vial	Yellow	BN00950-5

III. Storage and Handling:

Store kit at -20°C, protect from light. Let ALDH Assay Buffer warm to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions: (Read the entire protocol before proceeding)

ALDH Assay Buffer and Acetaldehyde: Store at -20 °C.

ALDH Substrate Mix: Reconstitute with 220 μl dH₂O. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.

ALDH Positive Control: Prepare 500 μl Assay Buffer containing 20% glycerol (Glycerol is not supplied). Reconstitute with 220 μl Assay Buffer containing 20 % glycerol. Pipette up and down to completely dissolve, aliquot and store at -20°C. Avoid repeated freeze/thaw cycle.

NADH Standard: Reconstitute with 500 μl dH₂O to generate 1 mM NADH. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.

V. ALDH Assay Protocol:

- NADH Standard Curve:** Add 0, 2, 4, 6, 8, 10 μl into a 96 well plate in duplicate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Adjust the volume to 50 μl/well with ALDH Assay Buffer.
- Sample Preparation:** Liquid samples can be measured directly. Tissue (50 mg) or cells (1 x 10⁶) should be rapidly homogenized with ~ 200 μl ice cold ALDH Assay Buffer for 10 minutes on ice, then spun down at 12000 rpm for 5 min to remove nuclei and insoluble material. Add 1 - 50 ul 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, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range. 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 use 10-20 μl, then adjust the final well volume to 50 μl with Assay Buffer.

- Reaction Mix:** Mix enough reagent for the number of samples and standards to be run: For each well, prepare a total 50 μl Reaction Mix containing:

	ALDH Measurement	Background Control
ALDH Assay Buffer	43 μl	48 μl
ALDH Substrate Mix	2 μl	2 μl
Acetaldehyde	5 μl	----

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

- Measurement:** Incubate at room temperature for 5 min and measure the OD of samples and sample backgrounds at 450 nm (A₁ & A_{1B}) then measure OD at 450nm (A₂ & A_{2B}) again after 20 - 60 min depending on the ALDH activity in the samples. The NADH standards can be measured at the end point. We suggest measuring the samples in a kinetic mode (every 2 - 3 min) and picking the linear range within the NADH Standard Curve.
- Calculation:** Subtract the 0 Standard reading from all Standard readings and plot the Standard Curve. Apply sample ΔOD 450nm [(A₂ - A_{2B}) - (A₁ - A_{1B})] to the Standard Curve to get B nmol of NADH generated during the reaction time (ΔT= T₂ - T₁).

$$\text{ALDH activity} = (B/(\Delta T \times V)) \times \text{Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

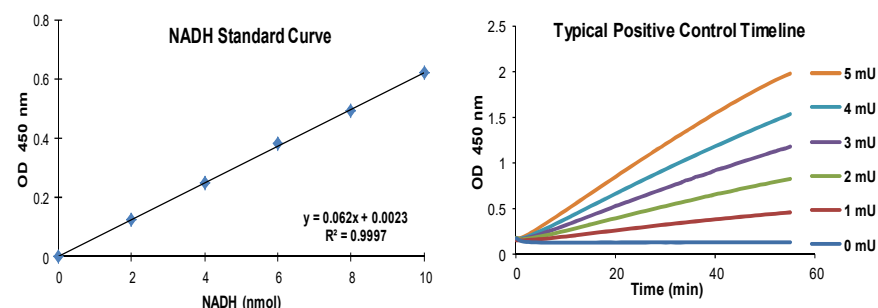
Where: **B** is the amount of NADH generated by your sample (nmol).

ΔT is the reaction time (min).

V is the sample volume used in the reaction well (ml).

Sample ALDH activities can also be expressed in mU/mg of sample, if total protein/ml is known.

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



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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		