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Aldehyde Dehydrogenase Activity Colorimetric Assay Kit

(Catalog #K731-100; 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 carboxyolic 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 BioVision 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	K731-100	Cap Code	Part Number
ALDH Assay Buffer	25 ml	WM	K731-100-1
Acetaldehyde	0.5 ml	Purple	K731-100-2
ALDH Substrate Mix (Lyophilized)	1 vial	Red	K731-100-3
ALDH Positive Control (Lyophilized)	1 vial	Green	K731-100-4
NADH Standard (0.5 µmol, Lyophilized)	1 vial	Yellow	K731-100-5

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 dH_2O. Pipette up and down to completely dissolve. Store at –20°C. Use within two months.
ALDH Positive Control: Reconstitute with 220 µl Assay Buffer containing 20 % glycerol (not included). Pipette up and down to completely dissolve, aliquot and store at -20°C. Avoid repeated freeze and thaw cycle.
NADH Standard: Reconstitute with 500 µl dH_2O to generate 1 mM NADH. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.

V. ALDH Assay Protocol:

1. **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.
2. **Sample Preparation:** Liquid samples can be measured directly. Tissue (50 mg) or cells (1 x 10^6) 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.
3. **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	----

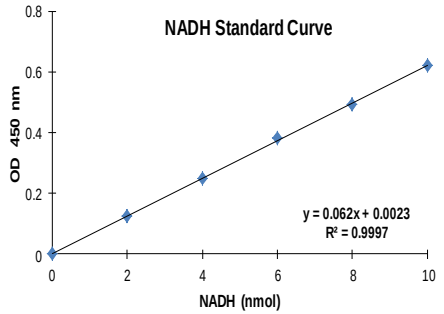
   Add 50 µl of the Reaction Mix to each well containing the Standard, test samples and background controls, mix well.
4. **Measurement:** Incubate at room temperature for 5 min and measure the OD of samples and sample backgrounds at 450 nm (A_1 & A_{1B}) then measure OD at 450nm (A_2 & A_{2B}) again after 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.
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings and plot the Standard Curve. Apply sample ΔOD 450nm [(A_2 - A_{2B}) - (A_1 - A_{1B})] to the Standard Curve to get B nmol of NADH generated during the reaction time (ΔT= T2 - T1).

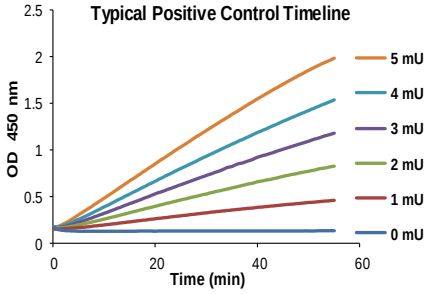
ALDH activity = (B/(ΔT X V)) x Dilution Factor = nmol/min/ml = mU/ml

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	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		

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