PicoProbe™ Aldehyde Dehydrogenase Activity Fluorometric Assay Kit

(Catalog #K741-100; 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 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. BioVision's PicoProbe™ Aldehyde Dehydrogenase Activity Assay Kit is a robust tool to quantify ALDH enzymatic activity. In this assay, acetaldehyde is oxidized by ALDH to form the NADH which couples with the PicoProbe to generate a potent fluorescence (Ex/Em = 535/587). The ALDH fluorometric assay kit is 10 times more sensitive than the ALDH colorimetric assay and can detect < 0.05 mU ALDH activity (based on our unit definition) in a variety of samples.

II. Kit Contents:

Components	K741-100	Cap Code	Part Number
ALDH Assay Buffer	25 ml	WM	K741-100-1
Acetaldehyde	0.5 ml	Purple	K741-100-2
PicoProbe™ (in DMSO)	0.4 ml	Blue	K741-100-3
ALDH Substrate Mix (Lyophilized)	1 vial	Red	K741-100-4
ALDH Positive Control (Lyophilized)	1 vial	Green	K741-100-5
NADH Standard (0.5 µmol, Lyophilized)	1 vial	Yellow	K741-100-6

III. Storage and Handling: (Read the entire protocol before proceeding)

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:

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

Aldehyde Dehydrogenase Substrate Mix: Reconstitute with 220 µl 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 Assay Buffer containing 20 % glycerol (not included). Pipette up and down to completely dissolve, aliquot & 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:

Dilute the NADH Standard to 0.05 mM by adding 10 µl of the NADH to 190 µl 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 pmol/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 pmol/well standards, adjust 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, dilute the reconstituted Positive Control 10-fold in Assay Buffer then use use 5 -10 µl and 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 performed: For each well, prepare a total 50 µl Reaction Mix containing:

	ALDH Measurement	Background Control
ALDH Assay Buffer	39 µl	44 µl
PicoProbe™**	2 µl	2 µl
Substrate Mix	2 µl	2 µl
Acetaldehyde	5 µl	----

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

****Note:** For NADH standard curve or samples which will generate less than 250 pmol NADH, reduce the probe volume to 1 µl per well to reduce reagent background and increase the assay buffer accordingly.

4. Measurement:

Incubate at room temperature for 5 minutes protected from light. Measure the RFU of samples and sample backgrounds at Ex/Em 535/587 nm (RFU_1 & RFU_{1B}) then measure the RFU at Ex/Em = 535/587 nm (RFU_2 & RFU_{2B}) again after 20 - 60 min depending on the ALDH activity in the samples. 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.

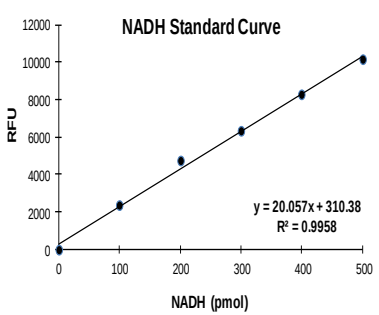
5. Calculation:

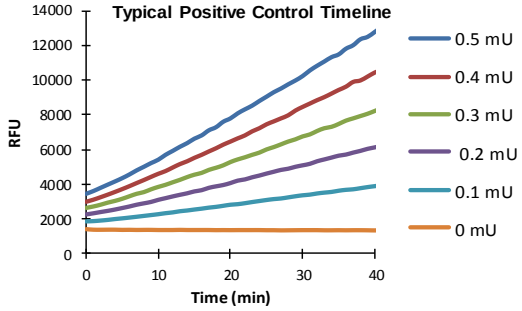
Subtract the 0 Standard reading from all Standard readings and plot the Standard Curve. Apply sample ΔRFU Ex/Em = 535/587 nm [(RFU_2 - RFU_{2B}) - (RFU_1 - RFU_{1B})] to the Standard Curve to get B nmol of NADH generated during the reaction time (ΔT= T2 - T1).

The ALDH activity = B/(ΔT X V) x Sample Dilution Factor = pmol/min/ml = µU/ml

Where: **B** is the amount of NADH generated in your sample (pmol)
ΔT is the reaction time (min)
V is a sample volume (ml)

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.





RELATED PRODUCTS:

ALDH Activity Colorimetric Assay Kit
Aspariginase Activity Assay Kit
Glucose Dehydrogenase Activity Assay Kit
Alcohol Dehrogenase Activity Assay Kit
Signal Transduction Pathway Products
Cytokines and Growth Factors
LDH Activity Assay Kit
Glutamate Dehydrogenase Activity Assay Kit
Isocitrate Dehydrogenase Activity Assay Kit
Stem Cell Fate Regulators
Protein Kinases
Metabolism Assay Kits

FOR RESEARCH USE ONLY! Not to be used on humans.

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.		