ab155894

PicoProbe ALDH

Activity Assay Kit

Instructions for Use

For the sensitive and accurate measurement of Aldehyde Dehydrogenase activity in cell and tissue culture supernatants, cell lysates, plasma, serum and other biological samples.

This product is for research use only and is not intended for diagnostic use.

Version 8b, Last Updated 7 July 2025

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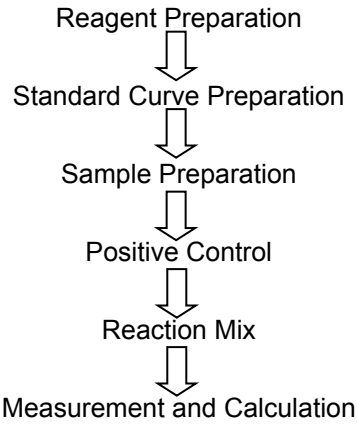
1. Overview

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.

Abcam's PicoProbe ALDH 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 28	25 mL
Acetaldehyde	0.5 mL
PicoProbe I	0.4 mL
Substrate Mix I	1 vial
ALDH Positive Control	1 vial
NADH Standard I	1 vial

* Store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

PLEASE NOTE: Assay Buffer 28 was previously labelled as Assay Buffer XXVIII and ALDH Assay Buffer. The composition has not changed.

Warm Assay Buffer 28 to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

- 96-well black plate with flat clear bottoms
- Multi-well spectrophotometer (ELISA reader)
- Glycerol

4. Assay Protocol

A. Reagent Preparation

1. Assay Buffer 28 and Acetaldehyde:
Store at -20°C.

2. Substrate Mix I:
Reconstitute with 220 µl Assay Buffer 28. Pipette up and down to completely dissolve. Store at –20°C. Use within two months

3. ALDH Positive Control:
Prepare 500 µl Assay Buffer 28 containing 20% glycerol (For example, add 100 µL glycerol to 400 µL Assay Buffer 28). Reconstitute with 110 µl assay buffer containing 20% glycerol. Pipette up and down to dissolve completely. Aliquot and store at -20°C.

4. NADH Standard I
Reconstitute with 500 µl dH_2O to generate 1 mM NADH. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.

B. ALDH Assay Protocol

1. NADH Standard Curve:

Dilute the NADH Standard I to 0.05 mM by adding 10 µl of the NADH to 190 µl Assay Buffer 28 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 Assay Buffer 28. 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 Assay Buffer 28.

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 Assay Buffer 28 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 Assay Buffer 28.

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 28 then use use 5 -10 µl and adjust the final well volume to 50 µl with Assay Buffer 28.

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
Assay Buffer 28	41 µl	46 µl
PicoProbe I**	2 µl	2 µl
Substrate Mix I	2 µl	2 µl
Acetaldehyde	5 µl	—

Add 50 µl of the Reaction Mix to each well containing the NADH Standard I, 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. Data Analysis

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

$$\textbf{ALDH Activity} = \frac{\textbf{B}}{\textbf{(}\Delta\textbf{T x V)}} \times \textbf{Sample Dilution Factor} = \textbf{pmol/min/ml = µU/ml}$$

Where:

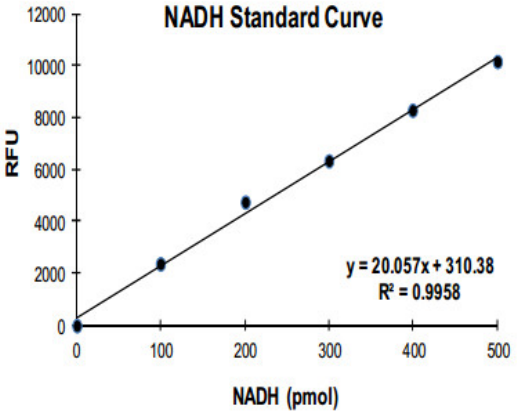
B is the amount of NADH generated in your sample (pmol).

ΔT is the reaction time (min).

V is the 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.

(a)



(b)

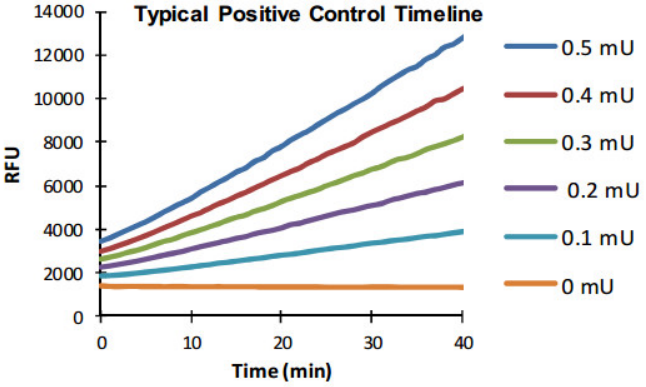


Figure 1: (a) NADH standard curve (b) Positive Control Timeline. Assays were performed following kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)

Problem	Reason	Solution
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix

Problem	Reason	Solution
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)