ab155893

ALDH Activity Assay Kit (Colorimetric)

Instructions for Use

For the sensitive and accurate measurement of ALDH activity in cell and tissue culture supernatants, cell lysates, plasma and serum.

View kit datasheet: www.abcam.com/ab155893
(use www.abcam.cn/ab155893 for China, or www.abcam.co.jp/ab155893 for Japan)

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

Version 7b Last Updated 30 May 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 ALDH Activity Assay Kit (Colorimetric) 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 < 0.1 mU of ALDH activity (based on our unit definition) in a variety of samples.

2. Protocol Summary

Reagent Preparation

Standard Curve Preparation

Sample Preparation

Positive Control

Reaction Mix

Measurement and Calculation

3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 28	25 mL
Acetaldehyde	0.5 mL
Developer Solution III	1 vial
ALDH Positive Control	1 vial
NADH Standard I	1 vial

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

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

Warm ALDH buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

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

4. Assay Protocol

A. Reagent Preparation

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

2. Developer Solution III:
Reconstitute with 220 µl dH_20. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

3. ALDH Positive Control:
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:

Add 0, 2, 4, 6, 8 and 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 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.

Note: 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 28.

Recommended input per well -
Biological fluids: 50 µL

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**
Assay Buffer 28	43 µl	48 µl
Developer Solution III	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. Data Analysis

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).

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

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.

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

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