

## PicoProbe™ Aldehyde Dehydrogenase Activity Assay Kit (Catalog #741-100; 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 hyperproliferemia, 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	1 ml	Blue	K741-100-2
PicoProbe™ (in DMSO)	1 vial	Purple	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:

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:

**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. 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<sub>2</sub>O 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<sup>6</sup>) 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 μl 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 5 - 10 μl and adjust the final well volume to 50 μl with Assay Buffer

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

### 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	4 μl	4 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 measure the RFU of samples and sample backgrounds at Ex/Em 535/587 nm (RFU<sub>1</sub> & RFU<sub>2a</sub>) then measure the RFU at Ex/Em 535/587 nm (RFU<sub>2</sub> & RFU<sub>2a</sub>) 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 450nm [(RFU<sub>2</sub> - RFU<sub>2a</sub>) - (RFU<sub>1</sub> - RFU<sub>1a</sub>)] to the Standard Curve to get B nmol of NADH generated during the reaction time (ΔT = T<sub>2</sub> - T<sub>1</sub>).

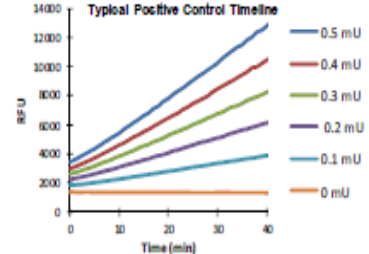
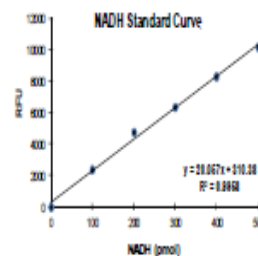
$$\text{The ALDH activity} = \frac{B}{(\Delta T \times V)} \times \text{Sample Dilution Factor} = \text{pmol/min/ml} = \mu\text{U/ml}$$

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.



### VI. RELATED PRODUCTS:

ALDH Activity Colorimetric Assay Kit  
Asparaginase Activity Assay Kit  
Glucose Dehydrogenase Activity Assay Kit  
Alcohol Dehydrogenase 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