

## Product Information

### Aldehyde Dehydrogenase Activity Colorimetric Assay Kit

Catalog Number **MAK082**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

Aldehyde dehydrogenases (ALDH) are a family of enzymes that catalyze the metabolism of exogenous and endogenous aldehydes, preventing the accumulation of potentially reactive and toxic aldehydes and their metabolites. In addition to their role in aldehyde metabolism, ALDH enzymes also play critical roles in other cellular processes such as cell proliferation, differentiation, and survival. Mutations in ALDH family members have been linked to numerous diseases such as heart disease, cataracts, and certain cancers. ALDH activity may also serve as a marker for certain stem cell populations including hematopoietic stem cells and certain cancer stem cells.

The Aldehyde Dehydrogenase Activity Colorimetric Assay kit provides a simple and direct procedure for measuring NAD-dependent ALDH activity in a variety of samples. ALDH activity is determined by a coupled enzyme assay in which acetaldehyde is oxidized by ALDH generating NADH, which reacts with a probe generating a colorimetric (450 nm) product proportional to the ALDH activity present. One unit of ALDH is the amount of enzyme that will generate 1.0  $\mu\text{mole}$  of NADH per minute at pH 8.0 at room temperature.

### Components

The kit is sufficient for 100 assays in 96 well plates.

ALDH Assay Buffer Catalog Number MAK082A	25 mL
Acetaldehyde Catalog Number MAK082B	0.5 mL
ALDH Substrate Mix Catalog Number MAK082C	1 vL
ALDH Positive Control Catalog Number MAK082D	1 vL
NADH Standard, 0.5 $\mu\text{mole}$ Catalog Number MAK082E	1 vL

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Glycerol (Catalog Number G6279 or equivalent)

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

ALDH Assay Buffer – Allow buffer to come to room temperature before use.

ALDH Substrate Mix– Reconstitute with 220  $\mu\text{L}$  of water. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at  $-20\text{ }^{\circ}\text{C}$ . Use within 2 months of reconstitution and keep cold while in use.

ALDH Positive Control – Assay Buffer + 20% Glycerol should be used to reconstitute the ALDH positive control, which the glycerol is a stabilizer for ALDH. We recommend portioning  $\sim 120\mu\text{L}$  of Assay Buffer and adding 30 $\mu\text{L}$  of glycerol, then using 110 $\mu\text{L}$  of this solution to reconstitute the positive control. The rest of the Assay Buffer should be free of glycerol. Mix well by pipetting, then aliquot and store, protected from light, at  $-20\text{ }^{\circ}\text{C}$ . Use within 2 months of reconstitution.

NADH Standard – Reconstitute with 500  $\mu\text{L}$  of water to generate a 1 mM NADH stock solution. Mix well by pipetting, then aliquot and store at  $-20\text{ }^{\circ}\text{C}$ . Use within 2 months of reconstitution.

**Storage/Stability**

The kit is shipped on wet ice and storage at  $-20\text{ }^{\circ}\text{C}$ , protected from light, is recommended.

**Procedure**

All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection

Add 0, 2, 4, 6, 8, and 10  $\mu\text{L}$  of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add ALDH Assay Buffer to each well to bring the volume to 50  $\mu\text{L}$ .

Sample Preparation

Tissue (50 mg) or cells ( $1 \times 10^6$ ) can be homogenized in 200  $\mu\text{L}$  of ice-cold ALDH Assay Buffer. Centrifuge the samples at  $13,000 \times g$  for 10 minutes to remove insoluble material.

Liquid samples can be measured directly.

**Note:** For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50  $\mu\text{L}$  with ALDH Assay Buffer.

**Notes:** NADH in the samples can generate a background signal. To remove the effect of NADH background, a sample blank may be set up for each sample by omitting the Acetaldehyde from the reaction mix.

For the positive control, add 10–20  $\mu\text{L}$  of the ALDH positive control solution to wells and adjust to 50  $\mu\text{L}$  with the ALDH Assay Buffer.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50  $\mu\text{L}$  of the appropriate Reaction Mix is required for each reaction (well).

**Table 1.**  
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
ALDH Assay Buffer	43 $\mu\text{L}$	48 $\mu\text{L}$
ALDH Substrate Mix	2 $\mu\text{L}$	2 $\mu\text{L}$
Acetaldehyde	5 $\mu\text{L}$	–

2. Add 50  $\mu\text{L}$  of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation
3. Incubate the plate at room temperature. After 5 minutes, take the initial measurement ( $T_{\text{initial}}$ ). Measure the absorbance at 450 nm at the initial time ( $A_{450\text{initial}}$ ).  
**Note:** It is essential ( $A_{450\text{initial}}$ ) is in the linear range of the standard curve.
4. Continue to incubate the plate at room temperature taking measurements ( $A_{450}$ ) every 2-3 minutes. Protect the plate from light during the incubation.
5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (10 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
6. The final measurement [ $(A_{450})_{\text{final}}$ ] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is  $T_{\text{final}}$ .  
**Note:** It is essential the final measurement falls within the linear range of the standard curve.

## Results

### Calculations

Correct for the background by subtracting the final measurement  $[(A_{450})_{\text{final}}]$  obtained for the 0 (blank) NADH standard from the final measurement  $[(A_{450})_{\text{final}}]$  of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the NADH standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the final sample blank value from the final sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of NADH present in the samples may be determined from the standard curve.

Using the corrected measurements, calculate the change in measurement from  $T_{\text{initial}}$  to  $T_{\text{final}}$  for the samples.

$$\Delta A_{450} = (A_{450})_{\text{final}} - (A_{450})_{\text{initial}}$$

Compare the  $\Delta A_{450}$  of each sample to the standard curve to determine the amount of NADH generated between  $T_{\text{initial}}$  and  $T_{\text{final}}$  (B).

The ALDH activity of a sample may be determined by the following equation:

$$\text{ALDH Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between  $T_{\text{initial}}$  and  $T_{\text{final}}$ .

Reaction Time =  $T_{\text{final}} - T_{\text{initial}}$  (minutes)

V = sample volume (mL) added to well

ALDH activity is reported as nmole/min/mL = milliunit/mL

One unit of ALDH is the amount of enzyme that will generate 1.0  $\mu\text{mole}$  of NADH per minute at pH 8.0 at room temperature.

Example:

NADH amount (B) = 5.84 nmole

First reading ( $T_{\text{initial}}$ ) = 3 minute

Second reading ( $T_{\text{final}}$ ) = 32 minutes

Sample volume (V) = 0.01 mL

Sample dilution is 1

ALDH activity is:

$$\frac{5.84 \times 1}{(32-3) \times 0.01} = 20.14 \text{ milliunits/mL}$$

**Troubleshooting Guide**

<b>Problem</b>	<b>Possible Cause</b>	<b>Suggested Solution</b>
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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