Aldehyde Dehydrogenase Activity Assay Kit

Item No. 700800

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

This kit arrives in two packages, due to different shipping temperatures. For best stability, Acetaldehyde should be stored at 4°C. However, if the acetaldehyde is frozen, it will be fine for one week at -20°C. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700014	HEPES Buffer (500 mM; pH 8.0)	1 vial/10 ml	-20°C
700805	ALDH Positive Control	2 vials/600 µg	-20°C
700802	Acetaldehyde Assay Reagent	1 vial/200 µl	4°C
700803	ALDH Enzyme Mixture	2 vials/400 µg	-20°C
700804	ALDH Cofactor	2 vials/3 mg	-20°C
700004	Fluorometric Developer Reagent	2 vials/60 µg	-20°C
700015	NADH Standard	2 vials/1.5 mg	-20°C
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

Acetaldehyde is carcinogenic. It is toxic if inhaled, ingested, or if in contact with skin. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes. Keep away from combustible materials.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
Email:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

Acetaldehyde should be stored 4°C as it will degrade over time at -20°C. Acetaldehyde has a stability of one week at -20°C. This kit will perform as specified if stored at -20°C and 4°C, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure fluorescence using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm
2. Adjustable pipettes and a multichannel pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. A 37°C incubator

INTRODUCTION

Background

Aldehyde dehydrogenases (ALDHs) represent a group of enzymes that oxidize a wide range of endogenous and exogenous aldehydes to their corresponding carboxylic acids.[1] Endogenous aldehydes are formed during the metabolism of amino acids, carbohydrates, lipids, biogenic amines, vitamins, and steroids. The human ALDH superfamily comprises 19 isozymes that possess important physiological and toxicological functions.[2,3] The ALDH1A subfamily plays a pivotal role in embryogenesis and development by mediating retinoic acid signaling.[2,3] The ALDH2 isozyme is predominantly linked with acetaldehyde detoxification in the second step of alcohol metabolism.[4,5] The amount of acetaldehyde to which cells or tissues are exposed after alcohol ingestion may be of great importance and may, among others, affect carcinogenesis.[6] ALDH1A1 and ALDH3A1 are lens and corneal crystallins, which are essential elements of the cellular defense mechanism against ultraviolet radiation-induced damage in ocular tissue.[7] ALDH1 activity has been shown to be increased in cancer stem cells and has been used to isolate cancer stem cells in different cancers.[8,9] Recently, pharmacological inhibitors have been developed for three of the 19 ALDH isozymes. These are the enzymes involved in the metabolism of alcohol (ALDH2) and the anticancer oxazaphophorine drugs (ALDH1A1 and ALDH3A1).[10] Increased or suppressed ALDH activity has been implicated in a variety of diseases, including cancer, Type II hyperprolinemia, Sjögren-Larsson Syndrome, Parkinson's Disease, cardiac disease, and hyperammonemia.[5,6,10,11] Therefore, the application of pharmacological inhibitors or activators of ALDHs represents a rational approach for the treatment of these pathological condition.

About This Assay

Cayman's Aldehyde Dehydrogenase Assay provides a fluorescence-based method for detecting ALDH activity in tissue homogenates, cell culture samples, and purified ALDH preparations. In the assay, ALDH catalyzes the oxidation of acetaldehyde to acetic acid, along with the concomitant reduction of NAD^+ to NADH. NADH reacts with the fluorometric developer to yield a highly fluorescent product which can be analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **HEPES Buffer (500 mM; pH 8.0) - (Item No. 700014)**

   This vial contains 10 ml of 500 mM HEPES, pH 8.0, and should be stored at -20°C. Mix the contents of the vial with 90 ml of HPLC-grade water. This final assay buffer (50 mM HEPES, pH 8.0) is used in the assay. The diluted assay buffer is stable for six months at 4°C.

2. **ALDH Positive Control - (Item No. 700805)**

   This vial contains lyophilized baker's yeast ALDH and should be stored at -20°C. Reconstitute the contents of the vial with 1.2 ml of diluted assay buffer and put the vial on ice. The reconstituted enzyme is stable for four hours at 4°C. Some activity is lost after freezing, so a second vial is included for additional assays.

3. **Acetaldehyde Assay Reagent - (Item No. 700802)**

   This vial contains 200 µl of 100 mM acetaldehyde and should be stored at 4°C. Mix 24 µl of acetaldehyde with 576 µl of HPLC-grade water to yield a 4 mM solution. This is sufficient reagent to assay 60 wells. Prepare additional assay reagent as needed. The diluted reagent is stable for one day at 4°C. The final acetaldehyde concentration in the well is 200 µM. If a lower concentration of acetaldehyde is desired, the 4 mM solution can be diluted further with HPLC-grade water.

4. **ALDH Enzyme Mixture - (Item No. 700803)**

   This vial contains a lyophilized powder of enzymes and should be stored at -20°C. Reconstitute the contents of the vial with 600 µl of diluted assay buffer and put the vial on ice. This is enough enzyme mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted enzyme mixture is stable for four hours at 4°C.

5. **ALDH Cofactor - (Item No. 700804)**

   This vial contains a lyophilized powder of NAD^+ and should be stored at -20°C. Reconstitute the contents of the vial with 600 µl of diluted assay buffer to yield an 8 mM solution of NAD^+. This is enough reagent to assay 60 wells. Prepare the additional vial as needed. The reconstituted reagent is stable for eight hours at 4°C.

6. **Fluorometric Developer Reagent - (Item No. 700004)**

   This vial contains a lyophilized powder of fluorometric developer and should be stored at -20°C. Reconstitute the contents of the vial with 600 µl of diluted assay buffer. This is sufficient reagent to assay 60 wells. Prepare the additional vial as needed. The reconstituted mixture is stable for one week at -20°C.

7. **NADH Standard - (Item No. 700015)**

   This vial contains a lyophilized powder of NADH and should be stored at -20°C. Reconstitute the contents of the vial with 2 ml of diluted assay buffer to yield a 1 mM solution of NADH. The reagent is ready to prepare the standard curve. The reconstituted standard is stable for four hours at 4°C.

Sample Preparation

Cell Lysate

1. Collect cells (~1.5 x 10^7 cells) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather, use a rubber policeman.
2. On ice, sonicate the cell pellet in 1 ml of cold buffer (*i.e.*, Tris or HEPES, containing protease inhibitors of choice; see **Interferences** on page 23).
3. Centrifuge at 10,000 x g for 10 minutes at 4°C. Remove the supernatant and store on ice.
4. If not assaying the same day, freeze at -80°C. The sample will be stable for one month when stored at -80°C.
5. Dilute the sample 1:2 with diluted assay buffer before assaying.

Tissue Homogenate

1. Prior to dissection, rinse the tissue with Tris or HEPES buffer to remove any red blood cells and clots.
2. Homogenize the tissue in 5-10 ml of cold buffer (*i.e.*, Tris or HEPES, containing protease inhibitors of choice; see **Interferences** on page 23) per gram weight of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.
5. Dilute the sample 1:2-1:10 with diluted assay buffer before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a NADH standard curve and ALDH positive control in duplicate needs to be assayed with the samples. We suggest that each sample be assayed at least in duplicate and in the presence and absence of acetaldehyde. A typical layout of standards, ALDH positive control, samples, and sample backgrounds to be measured in duplicate is given below in Figure 1. We suggest you record the contents of each well on the template sheet provided (see page 26).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17
B	B	B	B1	B1	B5	B5	B9	B9	B13	B13	B17	B17
C	C	C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18
D	D	D	B2	B2	B6	B6	B10	B10	B14	B14	B18	B18
E	E	E	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19
F	F	F	B3	B3	B7	B7	B11	B11	B15	B15	B19	B19
G	G	G	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20
H	PC	PC	B4	B4	B8	B8	B12	B12	B16	B16	B20	B20

A-G = Standards
PC = ALDH Positive Control
S1-S20 = Sample Wells
B1-B20 = Sample Background Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all wells.
- All reagents except the sample, ALDH positive control, and ALDH enzyme mixture must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all wells on the plate at one time.
- We recommend assaying samples at least in duplicate (triplicate preferred).
- The assay is performed at 37°C.
- Monitor the fluorescence with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Standard Preparation

Label seven polystyrene tubes A-G. Add the amount of reconstituted NADH (1 mM) and diluted assay buffer to each tube as described in Table 1. The diluted standards are stable for four hours at room temperature.

Tube	NADH (µl)	Assay Buffer (µl)	Concentration in well (µM)
A	0	1,000	0
B	20	980	1
C	40	960	2
D	80	920	4
E	120	880	6
F	160	840	8
G	200	800	10

Table 1. Preparation of NADH standards

Performing the Assay

1. **Standard Wells** - add 170 µl of diluted assay buffer, 10 µl of ALDH enzyme mixture, 10 µl of fluorometric developer reagent, and 10 µl of standard (tubes A-G) to the designated wells on the plate (see **Sample Plate Format**, Figure 1, page 12).
2. Read the fluorescence after five minutes at room temperature using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. Reading the standards prior to measuring the sample activity allows an appropriate *gain* to be established for detecting the entire range of standards. This *gain* must be used when assaying samples.
3. **ALDH Positive Control Wells** - add 150 µl of diluted assay buffer, 10 µl of ALDH cofactor, 10 µl of ALDH enzyme mixture, and 10 µl of ALDH positive control to at least two wells.
4. **Sample Wells** - add 150 µl of diluted assay buffer, 10 µl of ALDH cofactor, 10 µl of ALDH enzyme mixture, and 10 µl of sample to at least two wells.
5. **Sample Background Wells** - add 160 µl of diluted assay buffer, 10 µl of ALDH cofactor, 10 µl of ALDH enzyme mixture, and 10 µl of sample to at least two wells.
6. Add 10 µl of fluorometric developer reagent to all of the wells being used (sample, sample background, and ALDH positive control).
7. Initiate the reactions by adding 10 µl of acetaldehyde assay reagent to all positive control and sample wells. DO NOT add to sample background wells.
8. Read the fluorescence at 37°C every minute for 10-20 minutes using an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Reagent	Positive Control Wells (µl)	Sample Wells (µl)	Sample Background Wells (µl)
Diluted Assay Buffer	150	150	160
ALDH Cofactor	10	10	10
ALDH Enzyme Mixture	10	10	10
ALDH Positive Control	10	0	0
Sample	0	10	10
Fluorometric Developer Reagent	10	10	10
Initiate Reactions			
Acetaldehyde Assay Reagent	10	10	0

Table 2. Pipetting summary

ANALYSIS

Calculations

Plot the Standard Curve

1. Determine the average fluorescence of each standard. Subtract the fluorescence value of the standard A (0 µM) from itself and all other standards. This is the corrected fluorescence.
2. Plot the corrected fluorescence values (from step 1 above) of each standard as a function of the final concentration of NADH from Table 1. See Figure 2, on page 19, for a typical standard curve.

Determine the ALDH Activity

1. Determine the average fluorescence of each sample and sample background. Plot fluorescence as a function of time.
2. Determine the change in fluorescence (RFU) per minute by:

   a) Obtain the slope (rate) of the linear portion of the curve. An example of mouse liver homogenate is shown in Figure 3, on page 20.

   OR

   b) Select two points on the linear portion of the curve and determine the change in fluorescence during that time using the following equation:

$$\text{RFU/min} = \frac{\text{RFU (Time 2) - RFU (Time 1)}}{\text{Time 2 (min) - Time 1 (min)}}$$

3. Determine the rate (RFU/min) for the sample background and subtract this rate from the sample.
4. Calculate the ALDH activity using the equation below. One unit is defined as the amount of enzyme that will cause the formation of 1 nmol of NADH per minute at 37°C.

$$\text{ALDH Activity (nmol/min/ml)} = \left[\frac{\text{RFU/min}}{\text{Slope from standard curve (RFU/µM)}}\right] \times \frac{0.2\text{ ml}}{0.01\text{ ml}} \times \text{Sample dilution}$$

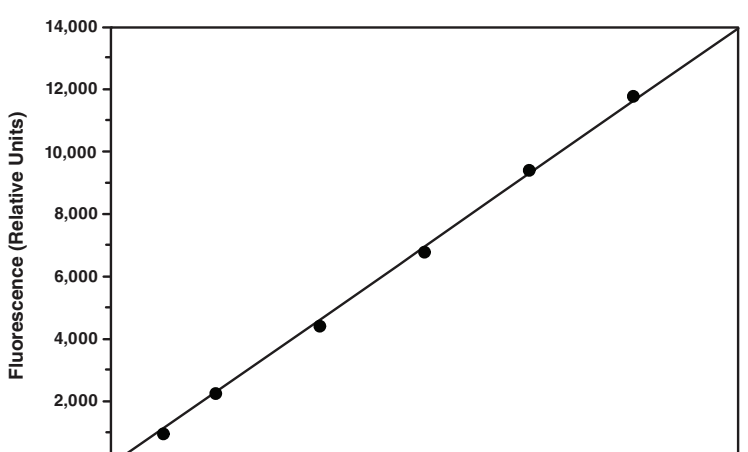

Figure 2. NADH standard curve

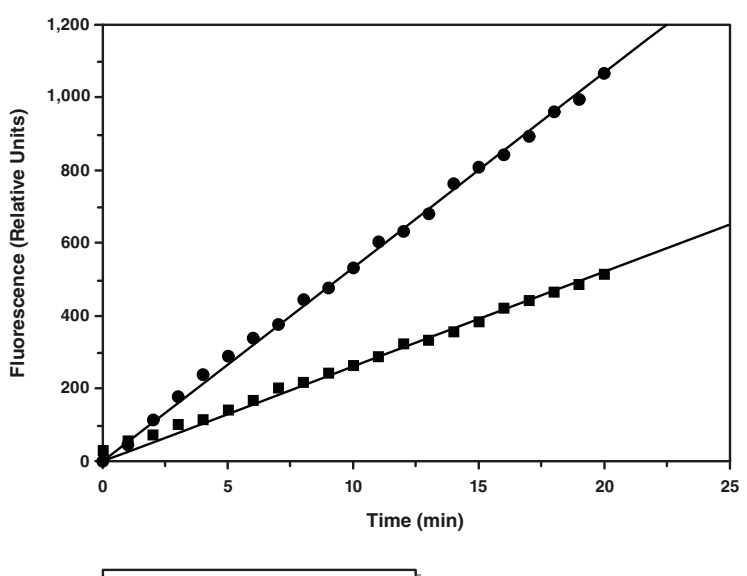

Figure 3. Reaction time course for mouse liver homogenate diluted 1:5 and 1:10

Mouse Liver Homogenate Dilution	RFU/min	ALDH Activity (nmol/min/ml)
1:5	52.3	9.1
1:10	24.3	8.4

Table 3. ALDH activity in mouse liver homogenate

Performance Characteristics

Precision:

When a series of sixteen mouse liver homogenate measurements were performed on the same day, the intra-assay coefficient of variation was 4.1%. When a series of sixteen mouse liver homogenate measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 3.9%.

Sensitivity:

The limit of detection for the assay is 0.4 U/ml (±0.1 U/ml) ALDH.

Interferences

The following reagents were tested in the assay for interference in the assay:

	Reagent	Will Interfere (Yes or No)
Buffers	50 mM Borate (pH 8.0)	Yes (63%)
	50 mM HEPES (pH 8.0)	No
	50 mM Phosphate (pH 7.6)	Yes (70%)
	1X Phosphate Buffered Saline (pH 7.4)	Yes (70%)
	50 mM Tris (pH 8.0)	No
Detergents	Polysorbate 20 (≤1%)	No
	Triton X-100 (≤0.1%)	No
Protease Inhibitors/ Chelators/ Enzymes	Antipain (10 µg/ml)	No
	Chymostatin (10 µg/ml)	No
	EDTA (≤1 mM)	No
	EGTA (≤1 mM)	No
	Leupeptin (10 µg/ml)	No
	PMSF (1 mM)	No
	Trypsin (10 µg/ml)	No
Solvents	Dimethylsulfoxide (5%)	No
	Ethanol (5%)	No
	Methanol (5%)	No
Others	BSA (0.1%)	No
	Dithiotreitol (≤1 mM)	No
	Glycerol (≤10%)	No
	NaCl (150 mM)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence detected above background in the sample wells	A. ALDH concentration is too low to detect B. The sample does not contain ALDH, or the sample contains something that is interfering	A. Re-assay using a more concentrated sample B. Check the **Interference** section for possible interference (see page 23)
The fluorometer exhibited 'MAX' values for the wells	The *gain* setting is too high	A. Reduce the *gain* and re-read B. Establish the *gain* with the NADH Standards prior to assaying samples
The fluorescence of the sample wells were higher than the last standard	A. ALDH in the sample was too high B. Sample was too concentrated	Dilute the samples with diluted assay buffer and re-assay; *NOTE: Remember to account for the dilution factor when determining ALDH activity*

References

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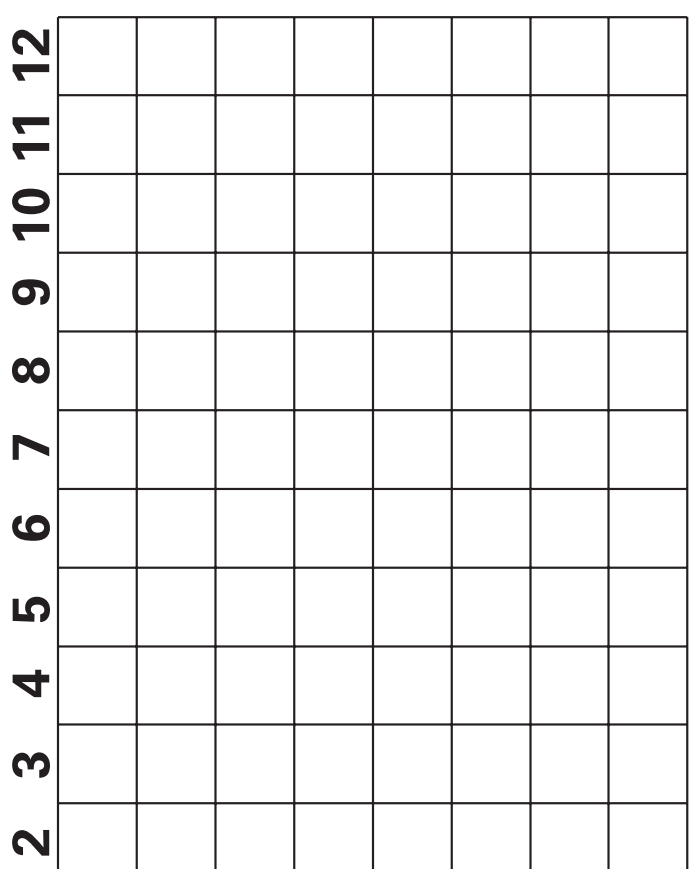

NOTES

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