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Product Information

Alcohol Dehydrogenase Activity Assay Kit

Catalog Number **MAK053**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Alcohol dehydrogenases (ADH) are a family of enzymes that catalyzes the conversion of alcohols to aldehydes, with the concomitant reduction of NAD^+ to NADH. In humans, there are nine isozymes of ADH, with the majority of ADH activity occurring in the liver. ADH family members are the primary enzymes involved in alcohol detoxification. Genetic variations in ADH enzymes result in differences in ADH activity and tolerances for alcohol, and may regulate susceptibility to alcoholism.

The Alcohol Dehydrogenase Activity Assay kit provides a simple and direct procedure for measuring ADH activity in a variety of samples. ADH activity is determined using ethanol as the substrate in an enzyme reaction, which results in a colorimetric (450 nm) product proportional to the enzymatic activity present. One unit of ADH is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 8.0 at 37 °C.

Components

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

ADH Assay Buffer Catalog Number MAK053A	25 mL
Ethanol, 2 M in water Catalog Number MAK053B	1 mL
Developer Catalog Number MAK053C	1 vl
ADH Positive Control Catalog Number MAK053D	1 vl
NADH Standard, 0.5 μmole Catalog Number MAK053E	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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the 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.

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

Developer – Reconstitute with 0.9 mL of water. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

ADH Positive Control – Reconstitute with 220 μL of ADH Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

NADH Standard – Reconstitute in 50 μL of water to generate a 10 mM NADH stock solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Dilute 10 µL of the 10 mM NADH stock solution with 90 µl of the ADH Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add ADH Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (50 mg) or cells (1×10^6) can be homogenized in 200 µL of ice-cold ADH Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to wells.

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 µL with ADH Assay Buffer.

For the positive control (optional), dilute 2 µL of the ADH Positive Control with 18 µL of ADH assay buffer. Add 2–10 µL of the diluted ADH positive control solution to wells and adjust to 50 µL with the ADH Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 100 µL of the appropriate Reaction Mix is required for each reaction (well).
   Note: NADPH and NADH in the samples will generate a background signal. To remove the effect of NADPH and NADH background, a sample blank may be set up for each sample by omitting ethanol.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
ADH Assay Buffer	82 µL	92 µL
Developer	8 µL	8 µL
2 M Ethanol	10 µL	–

2. Add 100 µL 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 37 °C. After 2–3 minutes, take the initial measurement ($T_{initial}$). Measure the absorbance at 450 nm at the initial time $(A_{450})_{initial}$.
   Note: It is essential $(A_{450})_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C taking measurements (A_{450}) every 5 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 $[(A_{450})_{final}]$ 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_{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 [$(A_{450})_{final}$] obtained for the 0 (blank) NADH standard from the final measurement [$(A_{450})_{final}$] 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.

Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

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

Compare the ΔA_{450} of each sample to the standard curve to determine the amount of NADH generated by the ADH assay between $T_{initial}$ and T_{final} (B).

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

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

B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.
Reaction Time = $T_{final} - T_{initial}$ (minutes)
V = sample volume (mL) added to well

ADH activity is reported as nmole/min/mL = milliunit/mL
One unit of ADH is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 8.0 at 37 °C.

Example:
NADH amount (B) = 5.84 nmole
First reading ($T_{initial}$) = 3 minute
Second reading (T_{final}) = 32 minutes
Sample volume (V) = 0.01 mL
Sample dilution is 1

ADH activity is:

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
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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