

Alcohol Dehydrogenase Activity Colorimetric Assay Kit

(Catalog #K787-100; 100 reactions; Store kit at -20°C)

I. Introduction:

Alcohol dehydrogenase (Alcohol DH, ADH) (EC 1.1.1.1) is a group of seven dehydrogenase enzymes that occur in many organisms and facilitate the interconversion between alcohols and aldehydes or ketones with the reduction of NAD⁺ to NADH. In humans and many other animals, they serve to break down alcohols which could otherwise be toxic; in yeast and many bacteria, some alcohol dehydrogenases catalyze the opposite reaction as part of fermentation. BioVision's Alcohol Dehydrogenase Assay Kit provides a convenient tool for sensitive detection of the ADH in a variety of samples. In the assay ADH utilizes ethanol as a substrate leading to a proportional color development. The activity of ADH can be easily quantified colorimetrically ($\lambda = 450 \text{ nm}$). This assay detects ADH activity as low as 0.01 mU in samples.

II. Kit Contents:

Components	K787-100	Cap Code	Part No.
ADH Assay Buffer	25 ml	WM	K787-100-1
Substrate	1 ml	Blue	K787-100-2
Developer (Lyophilized)	1 vial	Red	K787-100-3
ADH Positive Control (Lyophilized)	1 vial	Green	K787-100-4
NADH Standard (0.5 μmol , Lyophilized)	1 vial	Yellow	K787-100-5

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow ADH Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

- Reconstitute Developer with 0.9 ml of ddH₂O. Pipette up and down several times to completely dissolve the pellet into solution (**Do not vortex**).
- Reconstitute the ADH Positive Control with 220 μl Assay Buffer; Keep on ice during the preparation and protect from light. Aliquot and store -20°C.
- Reconstitute the NADH with 50 μl ddH₂O to generate a 10 mM NADH stock solution.
- The ADH Positive Control and the Developer are stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (< 5 times). Substrate & reconstituted NADH (10 mM) are stable for up to 6 months at -20°C.

V. Alcohol Dehydrogenase Assay Protocol:

- NADH Standard Curve:** Dilute 10 μl of the 10 mM NADH stock solution with 90 μl of ADH Assay Buffer to generate 1 mM NADH standard. Add 0, 2, 4, 6, 8, 10 μl of the 1 mM NADH standard into a 96-well plate in duplicate to generate 0, 2, 4, 6, 8, 10 nmol/well standards. Adjust the final volume to 50 μl with Assay Buffer.
- Sample Preparations:** Tissues (50 mg) or cells (1×10^6) can be homogenized in ~ 200 μl ice-cold Assay Buffer then centrifuged (13,000 x g, 10 min) to remove insoluble material. Add 2 - 50 μl samples into 96-well plate. For the positive control (optional), dilute Positive Control 1:9 by adding 2 μl of Positive Control to 18 μl Assay Buffer. Add 2-10 μl of diluted positive control solution to desired well(s). For serum sample, 5 - 50 μl serum can be directly tested. Adjust the final volume of test samples to 50 μl /well with Assay Buffer in the 96-well plate.

Notes:

A) We suggest testing several doses of your sample to make sure the readings are within the linear range of the standard curve.

B) NAD(P)H or other enzymes in samples may give non-specific readings, set up the background control (see next step below) to subtract the non-specific background interference in samples.

- Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a Reaction Mix (100 μl) containing:

Reaction Mix

82 μl ADH Assay Buffer
8 μl Developer
10 μl Substrate

Background Control Mix

92 μl ADH Assay Buffer
8 μl Developer

Add 100 μl of the Reaction Mix to each well containing the test samples, positive controls, and standards; add 100 μl of the Background Control Mix to each well containing the background control samples. Mix well.

- Measurement:** Incubate the mix for 3 min at 37°C, then measure OD at 450 nm in a microplate reader (A_0), incubate for another 30 mins to 2 hrs at 37°C and measure OD at 450 nm again (A_1), (incubation times will depend on the ADH activity in the samples). We recommend measuring the OD in a kinetic method (preferably every 3 - 5 min.) and choose the period of linear range within the standard curve to calculate the ADH activity of the samples. The NADH Standard Curve can read in Endpoint Mode (i.e., at the end of the incubation time).

- Calculation:** Subtract the 0 Standard value from all readings (standards and test samples). Plot NADH standard Curve. Calculate the OD increase by the test samples: $\Delta\text{OD} = A_1 - A_0$, apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by ADH during the reaction time ($\Delta T = T_2 - T_1$).

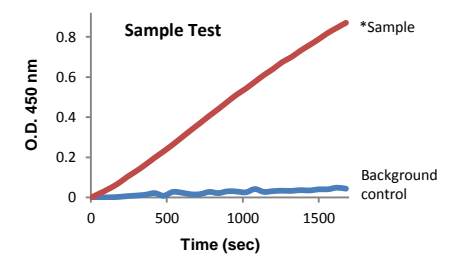
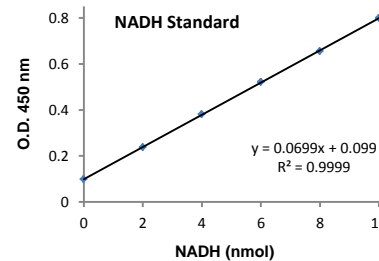
$$\text{ADH Activity} = \frac{B}{\Delta T \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: B is the NADH amount generated by ADH (in nmol).

T is the time of reaction (in minute).

V is the sample volume added into the reaction well (in ml).

Unit Definition: One unit is the amount of enzyme that will generate 1.0 μmol of NADH per min at pH 8 at 37°C.



*Sample: Bovine Liver extraction (36 μg protein)

RELATED PRODUCTS:

NAD/NADH Quantification Kit
Glucose Assay Kit
Ethanol Assay Kit
Lactate Assay Kit
L-amino Acid Assay Kit

NADP/NADPH Quantification Kit
ADP/ATP Ratio Assay Kit
Pyruvate Assay Kit
Lactate Assay Kit II
Glutamate Kit

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		