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ab102533

Alcohol Dehydrogenase Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Alcohol Dehydrogenase activity in various samples

View kit datasheet: www.abcam.com/ab102533
(use www.abcam.cn/ab102533 for China, or www.abcam.co.jp/ab102533 for Japan)

This product is for research use only and is not intended for diagnostic use.

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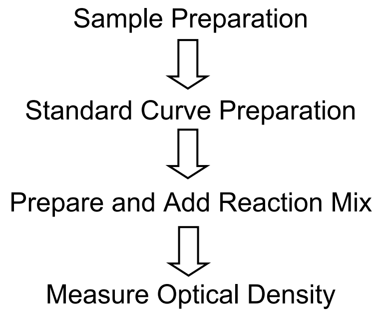
1. Overview

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 inter-conversion 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.

Abcam’s Alcohol Dehydrogenase Assay Kit provides a convenient tool for sensitive detection of the Alcohol DH in a variety of samples. In the assay Alcohol DH will utilize ethanol as a substrate leading to a proportional color development. The activity of ADH can be easily quantified colorimetrically (λ = 450 nm). This assay detects ADH activity as low as 0.01 mU in samples.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 34	25 mL
Substrate XII	1 mL
Developer Mix S	1 vial
ADH Positive Control	1 vial
NADH Standard I	1 vial

PLEASE NOTE: Assay Buffer 34 was previously labelled as Assay Buffer XXXIV and ADH Assay Buffer, and Developer Mix S as Developer VIII and Developer (Lyophilized). The composition has not changed.

* Store the kit at -20°C, protect from light.

- Allow Assay Buffer **34** to warm to room temperature before use.
- Briefly centrifuge vials prior to opening.
- Read the entire protocol before performing the assay.

DEVELOPER MIX S: Reconstitute Developer Mix S with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution. **(Do not vortex).**

ADH POSITIVE CONTROL: Reconstitute the ADH Positive Control with 220 µl Assay Buffer 34; Keep on ice during the preparation and protect from light. Aliquot and store -20°C.

NADH STANDARD I/NADH STANDARD: Reconstitute the NADH with 50 µl ddH2O to generate a 10 mM NADH stock solution.

- The ADH Positive Control and the Developer Mix S are stable for up to 2 months at -20°C after reconstitution or freeze-thaw cycles (< 5 times).
- Reconstituted NADH (10 mM) and the supplied Substrate XII solution are stable for up to 6 months at -20°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. **For tissue or cell samples:** Tissues (50 mg) or cells (1 x 10^6) can be homogenized in ~200 µl ice-cold Assay Buffer 34 then centrifuged (13,000 x g, 10 min) to remove insoluble material. Add 2-50 µl samples into 96-well plate.

b. **For serum/plasma samples:** 5-50 µl serum/plasma can be directly tested.

Note: Herapin or EDTA plasma is recommended

For the **positive control** (optional), dilute Positive Control 1:9 by adding 2 µl of Positive Control to 18 µl Assay Buffer 34. Add 2-10 µl of diluted positive control solution to desired well(s).

NAD(P)H or other enzymes in samples may give non-specific readings, set up the **background control** (see Step 3) to subtract the non-specific background interference in samples.

Adjust the final volume of test samples and positive control to 50 µl/well with Assay Buffer 34 in the 96-well plate.

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

2. Standard Curve Preparation:

Dilute 10 µl of the 10 mM NADH stock solution with 90 µl of Assay Buffer 34 to generate 1 mM NADH standard. Add 0, 2, 4, 6, 8, 10 µl of the 1 mM NADH Standard I 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 34.

3. 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	**Background Control**
Assay Buffer 34	82 µl	92 µl
Developer Mix S	8 µl	8 µl
Substrate XII	10 µl	--

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.

4. Measurement:

a) Incubate the mix for 3 min at 37°C, then measure OD at 450 nm in a microplate reader (A_0)

b) Incubate for another 30 mins to 2 hrs at 37°C and measure OD at 450 nm again (A_1).

Notes:

a) Incubation times will depend on the ADH activity in the samples.

b) We recommend measuring the OD in a kinetic method (preferably every 3-5 min.) and choosing 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).

5. Data Analysis

Subtract the zero Standard value from all readings (standards and test samples).

Plot NADH standard Curve.

Calculate the OD increase by the test samples: $\mathbf{\Delta OD = A_1 - A_0}$,

Apply the ΔOD to the NADH standard curve to get B nmol of NADH generated by ADH during the reaction time ($\Delta T = T_2 - T_1$).

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

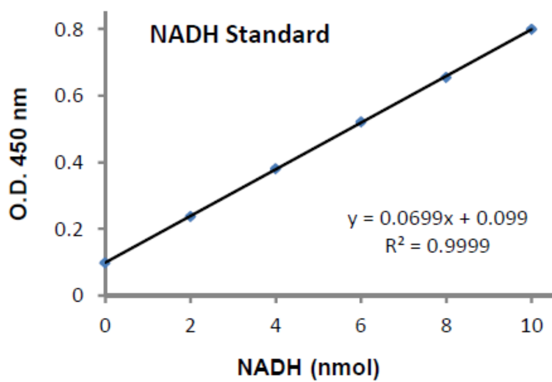
Where:

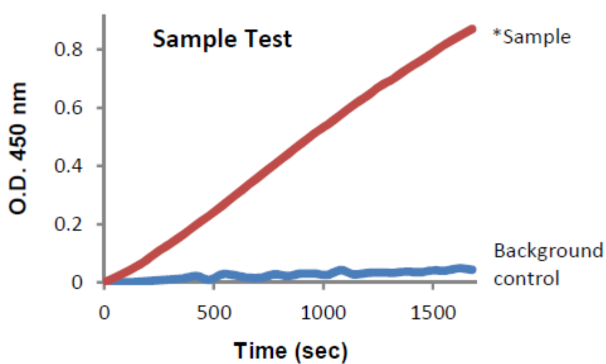
B is the NADH amount generated by ADH (in nmol).

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 (36µg protein)

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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