

Alcohol Dehydrogenase Activity Assay (Catalog: TBS2124, 100 Assays, Store at -20 °C)

DESCRIPTION

Alcohol dehydrogenase (ADH) belongs to the oxidoreductase enzyme family. ADH converts primary alcohols to aldehydes, simultaneously reducing NAD(P)⁺ to NAD(P)H. ADH activity is essential for healthy metabolism. Its dysfunction is associated with several metabolic conditions and diseases including alcoholism, alcoholic liver disease, aldehyde-related toxicities, neurodegenerative disorders, metabolic syndromes. Consequently, measuring ADH activity can help clinicians and researchers assess overall metabolic health and disease risk.

The Tribio™ Alcohol Dehydrogenase Activity Assay Kit utilizes a coupled dehydrogenase reaction that converts a chromophore into a colored formazan product, which is spectrophotometrically measured at an optical density (OD) of 460 nm. The intensity of the generated signal is directly proportional to the NAD(P)H content. This kit provides the easiest and most accurate approach to quantifying ADH activity across a wide variety of biological samples.

Synonyms: ADH, Aldehyde reductase, NAD(P)-dependent alcohol dehydrogenase, liver alcohol dehydrogenase.

APPLICATIONS

Specifically detect alcohol dehydrogenase from cell and biological tissues.

KIT CONTENTS FOR 100 TESTS

Name	Size (100 tests)
Assay Buffer	20 mL
Enzyme positive control (100x)	3 µL
Cofactor stock	420 µL
Enzyme substrate stock	120 µL
Developer	1.2 mL
NADPH Standard stock (2 mM)	0.5 mL

Storage conditions: Store the Reagent at -20°C.

PROCEDURES

1. Preparing working solutions:

- Dilute enzyme positive control by adding 1 µL of stock solution (100x) to 99 µL of assay buffer.
 - Prepare Substrate-cofactor working solution by adding 1 µL substrate stock, 4 µL of cofactor stock and 45 µL assay buffer for each well. Add 50 µL of working solutions to each well.
- Note:** A faster step is expected because the substrate is easy to vaporize.

2. Prepare NADPH Standards as Table 1.

3. Load samples: Add 40 µL of Standard or test samples, or blank (assay buffer) and enzyme positive control to the indicated well. (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

4. Add 50 µL of substrate-cofactor solution to the well of standards, test samples, enzyme positive control and blank.

Table 1: Preparation of NADPH Standards

Std#	NADH standard (µL)	Assay Buffer (µL)	NADH (µM)
1	200 (2 mM stock)	200	1000
2	200 (Std1)	200	500
3	200 (Std2)	200	250
4	200 (Std3)	200	125
5	200 (Std4)	200	62.5
6	200 (Std5)	200	31.25
7	200 (Std6)	200	15.625
8	Blank (0)	400	0

5. Add 10 µL of developer to each well, gently mix well.

6. Incubate at 37°C for about 30-60 minutes, with gentle shaking and protecting from light.

7. Measure OD460 nm. The standard and samples can be read at the end point.

8. Calculation using standard curve:

CALCULATION

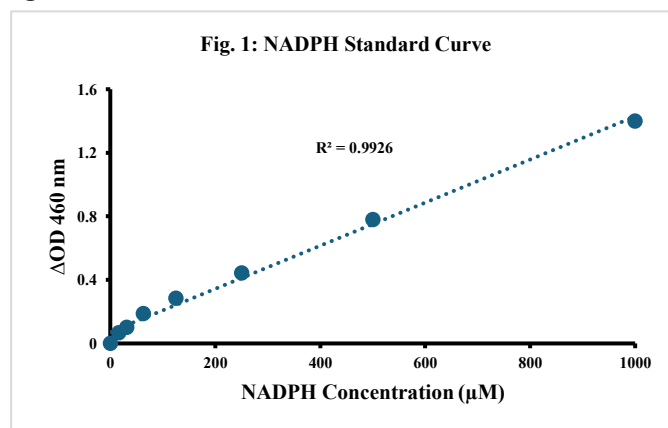
Subtract blank OD (Standard 0, #8) from the standard OD values and plot the ΔOD against standard concentrations. Determine the slope, and use the following equation to calculate the enzyme activity:

$$\text{ADH Activity (Unit/min)} = \text{DF} * (\text{OD SAMPLE} - \text{ODBLANK}) / (t * \text{Slope})$$

where OD SAMPLE is the measurement for each sample and OD BLANK is the OD value of the sample blank. Slope is the slope of the linear regression fits the standard points, and t is the reaction time (30 or 60 min). DF is the dilution factor.

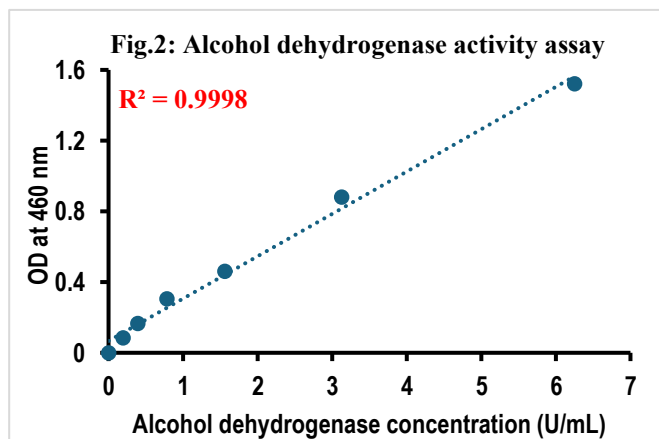
Unit definition: 1 Unit (U) will catalyze the conversion of 1 µM of non-colorimetric substrate to colorimetric product per min at 37°C.

Typical NADPH standard curve showed as an example in Fig.1.



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Typical Alcohol dehydrogenase activity assay curve showed as an example in Fig.2.



RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
 LDH Cytotoxicity Assay (TBS2002)
 Caspase-3 Colorimetric Assay kit (TBS2030)
 CD38 Cyclase Activity Assay (TBS2100)
 L-Lactate colorimetric assay (TBS2071)
 LDH Activity Assay (TBS2012)
 CCK-8 Cell Viability Assay (TBS2022)
 GOT Activity Assay (TBS2013)
 Thiol Fluorometric Assay (TBS2026)
 GSH Assay (TBS2028)
 Homocysteine Fluorometric Assay (TBS2091)
 AHCY Inhibitor Screening Assay (TBS2097)
 G6PDH Activity Colorimetric Assay (TBS2102)
 ATP Colorimetric/Fluorometric Assay (TBS2010)
 ADP Colorimetric / Fluorometric Assay (TBS2020)
 NNMT Inhibitor Screening Assay (TBS2097)
 NNMT Activity Assay (TBS2098)

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