Alcohol Dehydrogenase (ADH) Assay Kit

Catalog No.: abx298850

Size: 96 tests

Detection Range: 0.29 U/L – 248 U/L

Sensitivity: 0.29 U/L

Storage: Store all components in the dark at -20 °C

Application: For detection and quantification of ADH activity in serum, plasma, tissue homogenates, and other biological fluids.

Introduction

Abbexa's ADH Assay Kit is a quick, convenient, and sensitive method for measuring and calculating ADH activity. ADH catalyzes the dehydrogenation of ethanol, while concurrently NAD+ is reduced to NADH. WST-8 dye accepts electrons from NADH to produce a yellow-colored product, which has an absorbance maximum at 450 nm. The intensity of the color is proportional to ADH activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate Solution: 2 × 1.5 ml
3. Coenzyme: 2 vials
4. Coenzyme Diluent: 1 ml
5. Buffer Solution: 30 ml
6. Detection Reagent: 6 ml
7. Standard: 2 vials
8. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Normal saline (0.9% NaCl)
3. Pipette and pipette tips
4. Vials/tubes
5. Incubator (37 °C)
6. Centrifuge
7. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Collect using conventional methods, samples can be assayed directly.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml normal saline (0.9 % NaCl). Homogenize by hand in an ice water bath, using a mechanical homogenizer or by ultrasonication. Centrifuge the homogenate at 10,000 x g for 15 minutes, collect the supernatant and assay immediately. The protein content of the homogenate should be determined separately (abx097193). Repeat centrifugation if supernatant is cloudy.

Carrying out a preliminary experiment to determine the optimal dilution factor of samples is recommended before carrying out the formal experiment. Example dilutions are listed in the following table:

Sample Type	Dilution Factor
10 % Rat lung tissue homogenate	1
10 % Rat spleen tissue homogenate	1
10 % Rat liver tissue homogenate	2 - 4
10 % Rat heart tissue homogenate	1 - 3
10 % Rat brain tissue homogenate	1 - 3
10 % Mouse liver tissue homogenate	2 - 4
Mouse serum	1
Pig serum	1
Human serum	1
Dog serum	1

Notes:

- Sample diluent is normal saline (0.9% NaCl)
- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods.

2. Reagents

- **Coenzyme Solution:** Dissolve each vial in 400 µl Coenzyme Diluent, store at -20 °C up to 7 days.
- **Coenzyme Working Solution:** Dilute Coenzyme solution with Buffer Solution to a 1:99 ratio, store on ice and use within 30 minutes of preparation.
- **Reaction Working Solution:** Mix Coenzyme working solution with Substrate Solution to a 7:1 ratio, store on ice and use within 30 minutes of preparation.
- **5 mmol/L Standard Stock Solution**: Dissolve each vial in 1 ml Buffer Solution, store at -20 °C up to 5 days.
- **250 µmol/L Standard Solution:** Dilute standard stock solution with Buffer Solution to a 1:19 ratio, use within 6 hours of preparation.

B. Assay Procedure

1. **Standard curve preparation:** Label standard dilution tubes with 250, 200, 175, 150, 100, 75, 50, and 0 µmol/L. Dilute the 250 µmol/L standard solution with Buffer Solution as described in the following table:

Standard Concentration (µmol/L)	250 µmol/L standard solution (µl)	Buffer Solution (µl)
0	0	200
50	40	160
75	60	140
100	80	120
150	120	80
175	140	60
200	160	40
250	200	0

2. Add 20 µl of Standard Solution to the corresponding standard wells.
3. Add 20 µl sample to the corresponding sample wells.
4. Add 160 µl Reaction Working Solution and 40 µl Detection Reagent to each well and mix fully.
5. Incubate at room temperature for 2 minutes in the dark, then measure absorbances at 450 nm, record OD values as A_1.
6. Incubate at 37 °C for 15 minutes in the dark.
7. Measure absorbances at 450 nm, record OD values as A_2.

C. Calculation of Results

Standard curve is plotted with the OD values of $A_{2(Standard)}$ (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The ADH activity of the samples can be interpolated from the standard curve.

1. Serum and plasma samples:

One unit of ADH activity is defined as the quantity of ADH in 1 L of liquid sample that catalyzes the consumption of 1 µmol/L ethanol at 37°C per 1 minute.

$$\text{ADH activity (U/}L) = \frac{\Delta A_{450} - b}{a} \times \frac{f}{T}$$

2. Tissues homogenate samples:

One unit of ADH activity is defined as the quantity of ADH per 1 g of tissue protein content that catalyzes the consumption of 1 µmol/L ethanol at 37°C per 1 minute.

$$\text{ADH activity (U/g protein)} = \frac{\Delta A_{450} - b}{a} \times \frac{f}{T \times C_{pr}}$$

where:

y	$OD_{Standard} - OD_{Blank}$
x	Concentration of Standard
a	The slope of the standard curve
b	The intercept of the standard curve
ΔA_{450}	$A_2 - A_1$ for samples
T	Time of incubation reaction (15 minutes)
C_{pr}	Concentration of protein in tissue sample (gprot/L)
f	Sample dilution factor