Technical Bulletin

Alcohol Dehydrogenase Assay Kit

Catalogue Number MAK498

Product Description

Alcohol Dehydrogenase (ADH) is an oxidoreductase which catalyzes the interconversion of alcohols and aldehydes or ketones. ADH is important in humans and other organisms for the breakdown of alcohols which may otherwise be toxic. In yeast and some bacteria, ADHs catalyze the opposite reaction and produce alcohol as part of fermentation. The non-radioactive colorimetric ADH assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the enzyme activity.

The linear detection range of the kit is 0.4-80 U/L for a 30 minute reaction. The limit of detection is 0.1 U/L for a 120 min reaction. The kit is suitable for Alcohol dehydrogenase activity determination in biological samples such as plasma, serum, urine, tissue, and culture media.

Components

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

- Assay Buffer 10 mL
  Catalog Number MAK498A
- Diaphorase 120 µL
  Catalog Number MAK498B
- NAD solution 1 mL
  Catalog Number MAK498C
- MTT solution 1.5 mL
  Catalog Number MAK498D
- Substrate (10% Ethanol) 1 mL
  Catalog Number MAK498E
- Calibrator 1.5 mL
  Catalog Number MAK498F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories
  (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set
  (Catalog Number D9063 or equivalent)
- Phosphate Buffered Saline (PBS)
  (Catalog Number P3813 or equivalent)
- Potassium phosphate monobasic
  (Catalog Number P0662 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Assays can be executed at either desired temperature: 25 °C or 37 °C. Equilibrate all components to desired temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and plasma are assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize 50 mg of tissue in ~200 µL of buffer containing 50 mM potassium phosphate (pH 7.5)
3. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
4. Remove supernatant for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × g for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5)
4. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
5. Remove supernatant for assay.

All samples can be stored at –20 °C to –80 °C for at least one month.

Working Reagent

Note: Fresh reconstitution of the Working Reagent is recommended

Mix enough reagent for the number of assays to be performed. For each well, prepare 84 µL of Working Reagent or Blank Working Reagent according to Table 1.

Table 1

Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Substrate	5 µL	-
NAD Solution	9 µL	9 µL
MTT Solution	14 µL	14 µL
Diaphorase	1 µL	1 µL
Assay Buffer	55 µL	60 µL

Assay Reaction

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to wells should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Set the plate reader for the desired assay temperature (25 °C or 37 °C)
2. Transfer 100 µL of purified water (OD_{H2O}) and 100 µL of Calibrator (OD_{CAL}) solution into separate wells of a clear flat bottom 96-well plate.
3. Transfer 20 µL of each sample into 2 separate wells of the plate.
4. Add 80 µL of Working Reagent to one sample well (OD_S) and 80 µL of Blank Working Reagent to another sample well (OD_B). Tap plate lightly to mix.

Measurement

Read the optical density (OD) of each well at 565 nm immediately (OD_{0Min}) and again after 30 minutes (OD_{30}Min). Alternatively, using the plate reader's kinetic mode, monitor the OD for 30 minutes.

Results

1. Calculate ΔOD_S and ΔOD_B by subtracting the OD_0 value from the OD_{30} for each sample and sample blank well, respectively.
2. ADH activity can then be calculated as follows:

ADH Activity (U/L) =

$$\frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{MTT} \times l} \times \frac{\text{Reaction Vol (µL)}}{\text{Time x Sample Vol (µL)}} \times DF$$

$$= \frac{273}{t\ (min)} \times \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H2O}} \times DF(U/L)$$

where:

ε_{mtt} = The molar absorption coefficient of reduced MTT.
l = The light pathlength which is calculated from the calibrator.
OD_{CAL} = OD_{565}nm values of the Calibrator
OD_{H20} = OD_{565}nm values of the water
Time = The reaction time (30 minutes)
Reaction Vol = 100 µL
Sample Vol = 20 µL
DF = Sample dilution factor
(DF = 1 for undiluted Samples)

Note: If sample ADH activity exceeds 80 U/L, either use a shorter reaction time or dilute samples in purified water and repeat the assay. For samples with ADH activity < 1 U/L, the incubation time can be extended up to 120 minutes.

Unit definition: 1 Unit (U) of ADH will catalyze the conversion of 1 µmole of ethanol to acetaldehyde per min at pH 8.2.

Figure 1:

Typical ADH Assay Raw Kinetics Data

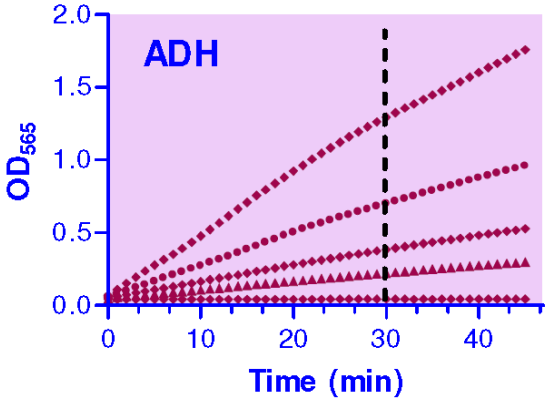
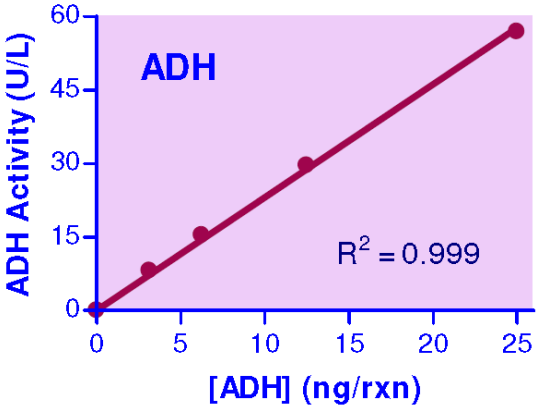


Figure 2:
Typical ADH Activity (30 minutes, 25 °C)



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mak498pis Rev 11/23