3050 Spruce Street, Saint Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

NAD–ADH Reagent Multiple Test Vial

Catalog Number **N7160**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonyms: Nicotinamide Adenine Dinucleotide–Alchohol Dehydrogenase Reagent

Product Description

Alcohol dehydrogenase (ADH) catalyzes the oxidation of alcohol to acetaldehyde with the simultaneous reduction of nicotinamide adenine dinucleotide (NAD) to NADH.[1] The consequent increase in absorbance at 340 nm is directly proportional to alcohol concentration in the sample.

$$\text{Ethanol} + \text{NAD} \xrightarrow{\text{ADH}} \text{Acetaldehyde} + \text{NADH}$$

Certain higher aliphatic alcohols will also react with ADH. Yeast ADH is most active with ethanol and its activity decreases as the size of the alcohol increases or decreases. Branched chain alcohols and secondary alcohols have very low activity. A comparison of relative reactivity with various alcohols follows:

Substance	Approximate Reactivity (%)
Ethanol	100
n-Butanol	40
Isopropanol	8
Methanol	0
Ethylene glycol	1
Acetone	0

Note that the relative reactivity is dependent upon the time at which the absorbency is measured. Increased interference may be observed with increase reaction times. In addition, certain drugs and other substances can influence ethanol levels.

Component

Each NAD–ADH Reagent multiple test vial contains ≥10 µmoles of NAD and ≥850 units of ADH (yeast).

ADH unit definition: One unit will convert 1.0 µmole of ethanol to acetaldehyde per minute at pH 8.8 at 25 °C.

Equipment and Reagents Required But Not Provided

- Spectrophotometer
- Cuvettes with optical properties suitable for use at 340 nm
- Pipettes
- Timer
- Centrifuge and stopper centrifuge tubes are needed for whole blood
- 0.5 M Glycine Buffer

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Add 16.0 ml of 0.5 M Glycine Buffer, pH 9, to the NAD–ADH Reagent Multiple Test Vial. Cap and invert several times to dissolve contents. DO NOT SHAKE. Allow the solution to reach room temperature before use.

0.5 M Glycine Buffer, pH 9 – Prepare the buffer by adding 37.5 g/l glycine (Catalog Number G7126) to water. Add 40-60 g/l sodium carbonate (Catalog Number 223530) to adjust the pH of the solution to 9.0±0.08.

Storage/Stability

Store the product at –20 °C.

The prepared NAD–ADH Reagent is stable at room temperature for 8 hours, 3 days if stored at 2–8 °C, and at least 2 months at –20 °C.

Procedures

Tests can be run on serum, plasma, or urine samples without sample preparation. Whole blood and markedly turbid samples should be deproteinized before the test is run.

Alcohol Determination for Serum, Plasma, or Urine

1. Label test tubes for BLANK and TESTS.
2. Add 3.0 ml of the prepared NAD–ADH Reagent to the labeled test tubes.
3. To BLANK tube add 0.01 ml (10 µl) water.
4. To TEST(s) add 0.01 ml (10 µl) of sample.
5. Cap or cover immediately and mix by gentle inversion.
6. Incubate solutions for 10 minutes at any temperature between 22–37 °C.
7. Transfer solutions to cuvettes and measure absorbance of TESTS at 340 nm vs BLANK. Complete readings within 10 minutes.

Alcohol Determination for Whole Blood, Turbid or Icteric Samples

1. Sample Deproteinization: Add 1.8 ml of 6.25% (w/v) trichloroacetic acid solution to centrifuge tube. While swirling tube, slowly add 0.2 ml of sample. Stopper tube immediately and mix by vortexing. Allow mixture to stand at room temperature for ~5 minutes.
   Note: If clumping of blood occurs, disperse clumps by crushing with a glass rod and remix.
2. Centrifuge at ~2000 rpm for 5 minutes to obtain a clear supernatant. Use this clear, protein-free supernatant as TEST sample.
3. Label test tubes for BLANK and TESTS.
4. Add 2.9 ml of the prepared NAD–ADH Reagent to the labeled test tubes.
5. To BLANK add 0.1 ml of water.
6. To TEST(s) add 0.1 ml of protein-free supernatant.
7. Cap or cover immediately and mix by gentle inversion.
8. Incubate solutions for 10 minutes at any temperature between 22–37 °C.
9. Transfer solutions to cuvettes and measure absorbance of TESTS at 340 nm vs BLANK. Complete readings within 10 minutes

Calculations

These calculations are for use with spectrophotometers that yield a linear response up to an absorbance of 1.5

Calculations based on 0.08% ethanol standard:

$$\text{Alcohol Concentration} = \frac{A_{340}\ \text{Sample}}{A_{340}\ \text{Standard}} \times \text{Concentration of ethanol std}$$

$$\text{Alcohol (mg/dl)} = \frac{A_{340}\ \text{Sample}}{A_{340}\ \text{Standard}} \times 80$$

Calculations based on absorbance at 340 nm:

$$\text{Alcohol (mg/dl)} = A_{340} \times 223$$

$$\text{Where: } 223 = \frac{3.01 \times 46 \times 100}{6.22 \times 0.01 \times 1 \times 1{,}000}$$

3.01 = Total reaction volume (ml)
46 = Molecular weight of ethanol
100 = Conversion of ml to dl
6.22 = Millimolar absorbtivity of NADH at 340 nm
0.01 = Volume of sample (ml)
1 = Lightpath of cuvette (cm)
1,000 = Conversion of ml to liter

Example: $A_{340} = 0.55$
Alcohol (mg/dl) = $0.55 \times 223 = 123$

Conversion Factors

To convert mg/dl to % (w/v), divide mg/dl results by 1,000. Thus, 123 mg/dl is = 0.123% (w/v).

To convert results to SI units (mmole/l), multiply mg/dl results by 0.217. Thus, 123 mg/dl = 26.7 mmole/l.

References

1. Lundquist, F., The determination of Ethyl Alcohol in Blood and Tissues. Methods of Biochemical Analysis, Vol. VII, Interscience (New York, NY: 1957) pp. 217-251.
2. Poklis, A., and Mackell, M.A., Evaluation of a modified alcohol dehydrogenase assay for the determination of ethanol in blood. Clin. Chem., **28**, 2125-2127 (1982).

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