



#### Intended Use

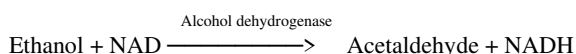
For **In-vitro Diagnostic** use in the automated, quantitative, determination of **Ethanol** in Serum or Plasma.

#### Clinical Significance (1-4)

An important toxicological problem in clinical diagnosis is Ethanol poisoning. Excess Ethanol ingestion produces a severe intoxication that may be fatal in some cases. Ethanol results in central nervous system depression. The metabolism of Ethanol proceeds through the formation of acetaldehyde.

#### Method Principle (5-6)

The Catachem Ethanol procedure is based on the affinity of an enzyme Alcohol Dehydrogenase from bacteria to catalyze the conversion of Ethanol to Acetaldehyde with the concomitant reduction of NAD to NADH. The increase in absorbance at 340 nm is directly related to the concentration of Ethanol in the sample.



#### Ethanol R1 (Sample diluent)

Each liter contains:

- Buffer
- Surfactants
- Stabilizer and nonreactive ingredients

#### Ethanol R2 (Activator)

- Buffer
- Alcohol dehydrogenase 400,000 U/L
- NAD 9 mM
- Surfactant
- Stabilizer and nonreactive ingredients

#### Precautions

Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

#### Reagent Storage And Stability

Store the Ethanol reagents at 2-8°C. When stored as directed, the reagents are stable until expiration date stated on the label.

Both reagent components are in a stabilized liquid format and are used as is. After uncapping each bottle the reagent is stable for 60 days if re-capped and stored in the refrigerator when not in use.

#### Reagent Indications Of Deterioration

- Turbidity
- Absorbance > 0.5 OD, 1 cm light path, 340nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed, contact Catachem technical service.

#### Specimen Collection and Stability

To maintain sample integrity and avoid changes in Ethanol concentrations care should be taken to collect the sample specimens:

Venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied.

Plasma specimens should be collected in tubes with heparin, sodium fluoride, EDTA, Citrate or oxalate as anticoagulants. Separate immediately from the cells and analyze promptly or store at 2-8°C.

#### Procedure

These instructions are outlined for performing the Catachem Ethanol assay using a manual procedure.

#### Materials Provided

- Catachem Ethanol Reagent
- Catachem Ethanol Calibrator material with assigned value
- Catachem Ethanol Quality Control material with assigned values

#### Materials Required But Not Provided

- Spectrophotometer equipped with 340nm wavelength

#### Calibration

Catachem protein-based Calibrator which contains a known Ethanol value is recommended.

#### Quality Control

To monitor the quality performance of the procedure used, Catachem Ethanol Control Level I and Control Level II should be included in the assay procedure.

#### Directions For Use

The Catachem Ethanol method uses two reagents R1 and R2

#### Analytical Parameters

|                         |             |
|-------------------------|-------------|
| Wavelength              | 340nm       |
| Temperature             | 37°C        |
| Pathlength              | 1 cm        |
| Reaction Mode           | End Point   |
| Reaction Time           | 5-6 minutes |
| Reagent Volume R1       | 0.500 ml    |
| Reagent Volume R2       | 0.100 ml    |
| Sample Volume           | 0.005 ml    |
| Total Volume            | 0.605 ml    |
| Sample-to-reagent ratio | 1:121       |

#### Assay Procedure

1. Bring the Ethanol R1 Reagent to room temperature.
2. Set spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing water.
3. Pipette 0.5 ml of Reagent into each of four cuvettes marked: "Sample" "Calibrator", "Control 1 and Control 2".
4. Incubate cuvettes for 3.0 minutes at 37°C.
5. Pipette 0.005 ml of calibrator, controls, and sample(s) into their respective cuvettes. Mix all cuvettes well.
6. Note the absorbance (A1).
7. Pipette 0.1 ml of R2 Reagent into each cuvette.
8. Incubate for 5 minutes.
9. Read absorbance after 5 minutes (A2)

10. Calculate the Ethanol concentration (mL/dL) in the sample(s), as shown in calculations and results.

### Calculations And Results

$$\text{Ethanol (mL/dL)} = \frac{\text{Sample (A2 - A1)}}{\text{Calibrator (A2 - A1)}} \times \text{Calibrator (mL/dL)}$$

**Example:**

|            | <u>A1</u> | <u>A2</u> |
|------------|-----------|-----------|
| Sample     | 0.20      | 0.3       |
| Calibrator | 0.20      | 0.5       |

$$\text{Calibrator} = 2.4 \text{ mL/dL (\%)}$$

$$\begin{aligned} \text{Ethanol (mg/dL)} &= \frac{0.3 - 0.2}{0.5 - 0.2} \times 2.4 \text{ mL/dL (\% vol/vol)} \\ &= 0.8 \text{ mL/dL (\% vol/vol)} \end{aligned}$$