DiscretPak™
ETHANOL REAGENTS C701-0A
FOR MANUAL OR AUTOMATED ASSAYS
FOR RESEARCH USE ONLY

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.

$$\text{Ethanol} + \text{NAD} \xrightarrow{\text{Alcohol dehydrogenase}} \text{Acetaldehyde} + \text{NADH}$$

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)

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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:

	A1	A2
Sample	0.20	0.3
Calibrator	0.20	0.5

Calibrator = 2.4 mL/dL (%)

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

= 0.8 mL/dL (% vol/vol)

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