CATACHEM

C701-0A

Ethanol Reagent Kit
IVDD, MHRA, CE
In the USA, for research use only and not for use in diagnostic procedures.

CONTENTS	PRODUCT NO.	PACKAGING
ETHANOL REAGENT KIT	**C701-0A**	
ETHANOL REAGENT (R1)	C701-01	2 X 13.0 mL
ETHANOL REAGENT (R2)	C701-02	2 X 2.6 mL
ETHANOL CALIBRATOR	C701-21	2 X 1.0 mL

INTENDED USE

The Catachem Ethanol Reagent Kit is intended for in vitro diagnostics use in the manual or automated, quantitative, determination of Ethanol in serum or plasma. This kit is intended for use in near-patient testing. The Ethanol Calibrator is intended for calibration in this procedure.

REAGENT PREPARATION

No preparation is required. The Catachem Ethanol reagents and calibrator are liquid and ready to use.

Instructions for use

1. Bring the liquid, ready-for-use Catachem Ethanol reagents to room temperature (15-30°C). Tilt the bottle to mix gently but thoroughly.
2. Proceed with use, following the instructions of the assay procedure and as directed by instrument manufacturer.
3. Following use, tightly cap vials and store unused material refrigerated between 2-8°C.
4. Dispose of materials in accordance with all applicable local and national regulations. Take care to align with your employer's chemical-specific and universal/standard precautions.

Reagent Storage and Stability

Once the Ethanol Reagent (R1) and Ethanol Reagent (R2) have been opened, they are stable at 2-8°C (refrigerated) for at least 60 days if tightly capped when not in use. Store the unopened Catachem Ethanol reagents and calibrator at 2-8°C (refrigerated). When stored as directed, the products are stable until the expiration date stated on the label. Once opened, the Catachem Ethanol Calibrator is stable for at least 60 days at 2-8°C. Products should be capped tightly between uses. Erroneous results may occur from prolonged exposure of opened vial to ambient air and/or elevated temperatures. Reagent or analyzer modifications may give a value other than that listed in assay data section.

Summary

The use of specific calibrators is an established procedure for optimal performance of manual or automated assay systems and is part of good laboratory practice. The Catachem Ethanol Calibration is a liquid, ready to use product that is intended for calibration use with the Catachem Ethanol Assay Method.

Intended Purpose

In the United States, for **research use only and not for use in diagnostic procedures.** NOT FOR USE IN UNPROFESSIONAL SETTINGS. For use in the automated, quantitative determination of Ethanol in serum or plasma. The Catachem Ethanol Calibrator is intended for use in calibration and this material is to be used with the Catachem Ethanol manual or automated quantitative assay of serum or plasma in accordance with the instrument manufacturer's directions.

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 340nm is directly related to the concentration of Ethanol in the plasma sample.

Reagents

Ethanol Reagent (R1) Sample Diluent
Each liter contains:
Buffer
Surfactants
Stabilizer and nonreactive ingredients

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Ethanol Reagent (R2) Activator
Each liter contains:

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

Ethanol Calibrator

Ethanol in buffer	20 mM/L (92 mg/dL)

Precautions
Handle these materials with gloves and personal protective equipment according to good laboratory practices as employed when handling live samples or any biological sample. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Contain and clean spills immediately. Dispose of materials according to local regulations and laws. Refer to SDS for additional information and determination of any residual risks. Calibrator material may contain tissue(s).

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 in the following ways:

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 tubed with heparin, sodium fluoride, EDTA, Citrate, or Oxalate as anticoagulants. Separate immediately from the cells and analyze promptly of store at 2-8°C (refrigerated).

Materials Provided
Catachem Ethanol Reagents (R1 and R2)
Catachem Ethanol Calibrator

Materials Required but Not Provided

- Spectrophotometer equipped with 340nm wavelength.

Calibration
Catachem's Ethanol Calibrator, which contains a known Ethanol value, is recommended.

Calibration Schedule
Calibration should be performed when this method is implemented for the first time and when quality control values fall outside of the indicated range.

Calibration Procedure
The Catachem Ethanol Calibrator is utilized by the analyst to check calibration of a manual or automated Catachem Ethanol reagent system in accordance with the instrument manufacturer's directions. Instructions for calibrating the automated analyzer/spectrophotometer are provided by the specific instrument manufacture. Read the entire recommended calibration procedure before proceeding with the instrument calibration.

Quality Control
To monitor the quality performance of the procedure, Catachem recommends the use of Catachem Catatrol Level 1 and Level 2, product no. C1200-11 and C1200-12, with assigned Ethanol values. These quality control materials should, if feasible, be included in the assay each time the procedure is performed.

Directions for Use
Catachem's Ethanol method requires two reagents.

Analytical Parameters

Wavelength	430nm
Temperature	37.0° C
Pathlength	1 cm
Reaction Mode	End point
Reaction Time	5-6 minutes
Reaction Volume (R1)	0.500mL
Reaction Volume (R2)	0.100mL
Sample Volume	0.005mL
Total Volume	0.605 m
Sample-to-reagent ratio	1:121

Assay Procedure

1. Ethanol reagents are ready to use. Bring the reagents to room temperature.
2. Set the spectrophotometer wavelength to 340nm, and zero the instrument with a reference cuvette containing water.
3. Pipette 0.5 mL of R1 Reagent into each of four cuvettes marked: "Blank", "Calibrator", "Sample 1 and Sample 2".
4. Incubate cuvettes for 3.0 minutes at 37°C

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5. Pipette 0.005mL of Calibrator, and sample(s) into their respective cuvettes. Mix all cuvettes well. Pipette 0.005mL of water into the "Blank cuvette".
6. Pipette 0.1mL of R2 Reagent into each cuvette
7. Incubate for 5 minutes.
8. Read absorbance after 5 minutes (A2) on all cuvettes.
9. Calculate the Ethanol concentration (mL/dL) in the sample(s), as show in calculations and results.

Reference Values

None detected ≤ 5 mg/dL

The value given here is only to be used as a guideline. It is recommended that each laboratory establish the normal range for the sample source and geographical area in which it is located.

Calculations and Results

Ethanol (mM/L) =

$$\frac{(\text{OD sample at 5 min.} - \text{OD Blank at 5 min.})}{(\text{OD Calibrator at 5 min.} - \text{OD Blank at 5 min.})} \text{ x Calibrator concentration}$$

Example:

Sample Absorbance – Blank = 0.44
Calibrator Absorbance – Blank = 0.88
Calibrator (mM/L) = 20.0

Ethanol (mM/L) = $\frac{0.44}{0.88} \text{ x } 20$

= 10mM/L

Interfering Substances

No materials should have a significant effect on the accuracy of this Ethanol procedure.

Method Performance Characteristics

Linearity: This procedure is linear over a range of 0-35mM/L.

Precision: Within-run and day-to-day precision is summarized below. Day to day precision was conducted over a 10-day period.

The following results were observed:

Ethanol	Within- Run Precision		Day-to-Day Precision	
Mean	SD	CV	SD	CV
mM/L	mM/L	%	mM/L	%
11.3	0.17	1.5	0.19	1.68
21.1	0.48	2.3	0.54	2.56
29.7	0.74	2.5	1.05	3.53

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