Ethylene Glycol Reagent Kit (including Calibrator and Bi-Level Controls)

C504-0A

IVDD, MHRA, CE

In the USA, for research use only and not for use in diagnostic procedures.

CONTENTS	PRODUCT NO.	PACKAGING
ETHYLENE GLYCOL KIT	**C504-0A**	
SAMPLE DILUENT (R1)	C504-01	2 x 10 mL
ACTIVATOR REAGENT (R2)	C504-02	1 x 8.5 mL
ETHYLENE GLYCOL CALIBRATOR	C504-10	1 x 3 mL
ETHYLENE GLYCOL CONTROL LEVEL I	C504-11	1 x 3 mL
ETHYLENE GLYCOL CONTROL LEVEL 2	C504-12	1 x 3 mL

CALIBRATOR AND CONTROLS

ETHYLENE GLYCOL CALIBRATOR **STORAGE:** 2-8°C **LOT NO:** XX000000
C504-10 (1 x 3 mL) **EXPIRATION DATE:** YYYY-MM-DD

CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS	CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS	S.I. UNITS
Ethylene Glycol Calibrator	Kinetic Rate UV Method	155	mg/dL	25	mmol/L

ETHYLENE GLYCOL CONTROL LEVEL 1 **STORAGE:** 2-8°C **LOT NO:** XX000000
C504-11 (1 x 3 mL) **EXPIRATION DATE:** YYYY-MM-DD

CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS		CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS		S.I. UNITS
		Mean	Range		Mean	Range	
Ethylene Glycol Control Level 1	Kinetic Rate UV Method	56	46-66	mg/dL	9.00	7.41-10.63	mmol/L

ETHYLENE GLYCOL CONTROL LEVEL 2 **STORAGE:** 2-8°C **LOT NO:** XX000000
C504-12 (1 x 3 mL) **EXPIRATION DATE:** YYYY-MM-DD

CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS		CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS		S.I. UNITS
		Mean	Range		Mean	Range	
Ethylene Glycol Control Level 2	Kinetic Rate UV Method	248	208-288	mg/dL	40.00	33.51-46.40	mmol/L

Assigned values, listed in the above tables, were obtained from multiple assays over a period of seven days on an automated analyzer. Verify that the product lot number on the data sheet corresponds to the lot number printed on the vial label. Ethylene Glycol Calibrator and Controls contain bovine serum. This kit is non-sterile and single use.

INTENDED USE

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

REAGENT PREPARATION

1. Add exactly 10 mL of distilled or deionized water to a bottle of Sample Diluent (R1). Mix gently by inversion. The reconstituted reagent is now ready for use.
2. The Activator Reagent (R2) is packaged ready for use. No preparation is required.
3. The Ethylene Glycol Calibrator, Control Level 1, and Control Level 2 are liquid, ready to use, purified protein-based preparations to which chemical components have been added to attain selected constituent levels. Additives are included to preserve the integrity of the products. The products are packaged ready for use. No preparation is required.

Instructions for use

1. Bring the liquid, ready-for-use Catachem Ethylene Glycol Calibrator, Controls Level 1 and Level 2 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 Ethylene Glycol Sample Diluent (R1) has been reconstituted, it is stable at 2-8°C (refrigerated) for 30 days if tightly capped when not in use. After opening, the Ethylene Glycol Activator Reagent (R2) is stable at 2-8°C (refrigerated) for 60 days if tightly capped when not in use. Store the unopened Catachem Ethylene Glycol reagents, calibrator, and controls at 2-8°C (refrigerated). When stored as directed, the reagents are stable until the expiration date stated on the label.
Once opened, the Catachem Ethylene Glycol Calibrator and Controls Level 1 and Level 2 are 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.

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Ethylene Glycol Reagent Kit (including Calibrator and Bi-Level Controls)

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Summary

The use of specific calibrators and assayed quality control materials is an established procedure for optimal performance of manual or automated assay systems and is part of good laboratory practice. The Catachem Ethylene Glycol Calibrator, Control Level 1 and Level 2 are liquid, ready to use products that have been specifically formulated for use with the Catachem Ethylene Glycol 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 Ethylene Glycol in serum or plasma. Catachem Ethylene Glycol Calibrator and bi-level Controls are intended for use in calibration and as assayed quality control material. These materials are to be used with the Catachem Ethylene Glycol manual or automated quantitative assay of serum or plasma in accordance with the instrument manufacturer's directions.

Clinical Significance (1-2)

An important toxicological problem in clinical diagnosis is Ethylene Glycol poisoning. When ingested in the form of antifreeze or other industrial products, Ethylene Glycol results in central nervous system depression, cardiopulmonary compromise, and renal insufficiency. Laboratory features of Ethylene Glycol poisoning include increased anion gap, increased osmolal gap, calcium oxalate crystalluria, and detectable Ethylene Glycol in serum.

Method Principle (3-4)

The Catachem Ethylene Glycol procedure is based on the affinity of a specific Glycerol Dehydrogenase (from a bacterial source) to catalyze the oxidation-reduction reaction of Ethylene Glycol in the presence of NAD. This two-point kinetic procedure is read at 340 nm and the increase in rate of absorbance is directly proportional to the concentration of Ethylene Glycol in the serum sample.

$$Ethylene\ Glycol + NAD \xrightarrow{GDH} NADH + Glycoaldehyde + H^+$$

Sample Diluent

Each liter contains:

Buffer	
Glycerol Dehydrogenase	≥1000 Units
Stabilizer and non-reactive ingredients	

Activator Reagent

NAD	5.0 mmol
Stabilizer and non-reactive ingredients	

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, controls, and linearity materials contain tissues.

Reagent Indications of Deterioration

- Turbidity
- Absorbance > 0.8 OD, 1 cm light path, 340 nm
- Quality control values out of assigned ranges

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

Specimen Collection and Stability (1)

To maintain sample integrity and avoid changes in Ethylene Glycol concentrations, care should be taken to collect the specimen sample.

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 cells and analyze promptly or store at 2-8 °C for up to 12 hours.

Materials Provided

Catachem Ethylene Glycol Reagents

Catachem Ethylene Glycol protein-based Calibrator and Controls with assigned values

Materials Required but Not Provided

Automated analyzer/spectrophotometer equipped with 340nm wavelength.

Calibration

Catachem's protein-based calibrator which contains a known Ethylene Glycol value is recommended.

Calibration Schedule

Calibration should be performed when the method is implemented on the automated analyzer/spectrophotometer for the first time. Recalibration is required after changes of reagent, lot number, major instrument service, and when quality control values are out of the indicated range.

Calibration Procedure

The Catachem Ethylene Glycol Calibrator is utilized by the analyst to check calibration of a manual or automated Catachem Ethylene Glycol reagent system

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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
The Catachem Ethylene Glycol Control Level 1 and Level 2 are used to verify calibration and performance of the Catachem Ethylene Glycol reagent system in accordance with the instrument manufacturer's directions. To monitor the quality performance of the procedure used, Catachem Ethylene Glycol Control Level 1 and Control Level 2 should be included in the assay procedure when the assay is run.

Directions for Use
Catachem's Ethylene Glycol method requires two reagents.

Procedure Limitations
Samples with ethylene glycol values greater than 300 mg/dL should be diluted 1:2 with physiological saline and re-assayed. Multiply results obtained by 2 to adjust for the sample dilution.

Materials Required (Not Provided)
Spectrophotometer
Cuvettes 1 cm light path

Timer	to time incubation time
Pipette	0.5mL and 0.1 mL for reagents
Pipette	0.006mL for sample

Materials Provided
Enzyme Reagent
Activator Reagent
Calibrator
Quality Controls Level I and Level II

Analytical Parameters

Wavelength	340 nm
Temperature	37°C
Pathlength	1 cm
Reaction Mode	Rate
Reaction Time	5-10 minutes
Reaction Volume (R1)	0.500 mL
Reaction Volume (R2)	0.100mL
Sample Volume	0.006mL
Total Volume	0.606 mL
Sample-to-reagent ratio	1:101

Assay Procedure
1. Bring the Ethylene Glycol Working Reagent to room temperature.
2. Set the spectrophotometer wavelength at 340 nm and zero the instrument with the cuvette containing water.
3. Pipette 0.5 mL of R1 Reagent into each of four cuvettes marked: "sample", "calibrator", "control 1", "control 2".
4. Pipette 0.006 mL of calibrator, controls and sample(s) into their respective cuvettes. Mix all cuvettes well.
5. Incubate cuvettes for 3.0 minutes at 37°C.
6. Pipette 0.100 mL of Activator Reagent into all cuvettes. Mix all cuvettes well and incubate for 2 minutes. After this 2-minute period take an initial read (A1) and then after an additional 2 minutes take a second read (A2) to obtain a ∆-absorbance (OD @ 340 nm)
7. Read the ∆-absorbance for "calibrator", "control 1", "control 2", and "sample".
8. Calculate the Ethylene Glycol concentration (mg/dL) in the sample(s), as shown in calculations and results.

Calculations and Results

$$Ethylene\ Glycol\left(\frac{mg}{dL}\right) = \frac{\Delta OD\ Sample\ (A2-A1)}{\Delta OD\ Calibrator\ (A2-A1)} * Calibrator\left(\frac{mg}{dL}\right)$$

Example:	A1	A2
Sample	0.01	0.13
Calibrator	0.05	0.15

Calibrator =150 mg/dL

$$Ethylene\ Glycol\frac{mg}{dL} = \frac{0.13-0.01}{0.15-0.05} * 150\frac{mg}{dL} = 90\frac{mg}{dL}$$

Reference Values (5)

None detected	≤ 5 mg/dL
Potentially Toxic	>100 mg/dL

Interfering Substances
The following substances have no significant effect on the accuracy of this Ethylene Glycol procedure at the concentrations stated.

- Glycerol ≤ 47 mg/dL
- Hemoglobin ≤ 200 mg/dL
- Triglycerides ≤ 1000 mg/dL
- Bilirubin ≤ 2.2 mg/dL
- Propylene glycol ≤ 100 mg/dL
- Ethanol (76mM) ≤ 350 mg/dL
- Fomepizole (4 methyl pyrazole) ≤ 120 mg/L
- Acetaminophen ≤ 1,000 µg/mL

- Lactate 200mM + Lactate Dehydrogenase (LDH at 12,000U/L)

Other alcohols and associated materials have been evaluated to determine potential interferences with this assay and are referenced below (6). Certain drugs are also known to influence the Ethylene Glycol Values (1-2).

Method Performance Characteristics

Linearity: This procedure is linear over a range of 0-300 mg/dL (0-48 mmol/L).

Limit of Detection: 1.1 mg/dL (as determined on a Beckman AU 480)

Limit of Quantification: 8.6mg/dL (as determined on a Beckman AU 480)

Precision: Precision data was obtained using five levels of protein-based controls on a Beckman AU480.

The following results were observed:

Ethylene Glycol	Within- Run Precision		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
6	0	0	0	0
88	0	0	3.339	3.940
155	2.920	1.886	3.879	2.502
224	2.610	1.165	4.903	2.188
255	2.619	1.027	9.577	3.755

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