



C604-0A

Methanol Reagent Kit
 IVDD, MHRA, CE

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

CONTENTS	PRODUCT NO.	PACKAGING
METHANOL KIT	C604-0A	
METHANOL REAGENT (R1)	C604-01	2 x 10 mL (Lyo)
METHANOL REAGENT DILUENT (R2)	C604-02	2 x 10 mL
METHANOL CALIBRATOR	C604-10	1 x 3 mL
METHANOL CONTROL LEVEL 1	C604-11	1 x 3 mL
METHANOL CONTROL LEVEL 2	C604-12	1 x 3 mL

CALIBRATOR AND CONTROLS

METHANOL CALIBRATOR C604-10 (1 x 3 mL)		STORAGE: 2-8°C		LOT NO: XX000000 EXPIRATION DATE: YYYY-MM-DD	
CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS	CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS	S.I. UNITS
Methanol	End Point Two-Point	1,000	mg/L	31.21	mmol/L

METHANOL CONTROL LEVEL 1 C604-11 (1 x 3 mL)		STORAGE: 2-8°C		LOT NO: XX000000 EXPIRATION DATE: YYYY-MM-DD		
CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS		CALIBRATION VALUE S.I. UNITS		S.I. UNITS
		Mean	Range	Mean	Range	
Methanol	End Point Two-Point	500	400 - 600	15.60	12.48 - 18.73	mmol/L

METHANOL CONTROL LEVEL 2 C604-12 (1 x 3 mL)		STORAGE: 2-8°C		LOT NO: XX000000 EXPIRATION DATE: YYYY-MM-DD		
CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS		CALIBRATION VALUE S.I. UNITS		S.I. UNITS
		Mean	Range	Mean	Range	
Methanol	End Point Two-Point	1,500	1,200 - 1,800	46.82	37.45 - 56.18	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. Methanol Calibrator and Controls contain bovine serum. This kit is non-sterile and single use.

INTENDED USE

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

REAGENT PREPARATION

1. Prepare Catachem Methanol Working Reagent by pouring the entire contents (10mL) of Reagent Diluent (R2) (C604-02) into the Methanol Reagent bottle (C604-01).
2. Mix gently by inverting the bottle for 3-5 minutes.
3. The Methanol Calibrator, Control Level 1, and Control Level 2 are packaged ready for use, with no preparation required.

Instructions for use

1. Bring the prepared Methanol Working Reagent (combined C604-01 and C604-02), the Calibrator (C604-10) and each control (C604-11 and C604-12) to room temperature (15-30°C).
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

Store the unopened Methanol Reagents at 2-8°C (refrigerated). When stored as directed, these reagents are stable until the expiration date stated on the label. Store the unopened Methanol Calibrator, Control 1, and Control 2 at 2-8°C (refrigerated). When stored as directed, these products are stable until the expiration date stated on the label. After preparation, the Methanol Working Reagent (combined C604-01 and C604-02) is stable at 2-8°C (refrigerated) for 30 days. After opening, the Methanol Calibrator, Control Level 1, and Control 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.



Catachem, Inc., 353 Christian Street, Suite 2, Oxford, Connecticut, 06478 USA • Phone: (203) 262-0330 • catachem@catacheminc.com

NuuChem Diagnostics, LTD., 18 Killuney Road, Armagh, Northern Ireland, BT61 9HR • trevor.templeton@nuuchem.com



C604-0A

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

Methanol Reagent Kit
IVDD, MHRA, CE

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 Methanol Calibrator, Control Level 1 and Level 2 are liquid, ready to use products that have been specifically formulated for use with the Catachem Methanol Assay Method.

Intended Purpose

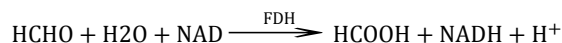
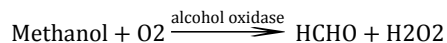
In the United States, for **research use only and not for use in diagnostic procedures**. NOT FOR USE IN UNPROFESSIONAL SETTINGS. For use in the automated, quantitative determination of methanol in serum or plasma. Catachem Methanol Calibrator and bi-level Controls are intended for use in calibration and as assayed quality control material. These materials are to be used with the Catachem Methanol 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 Methanol poisoning. Methanol ingestion produces a severe intoxication that may be fatal in many cases. Methanol results in central nervous system depression. The metabolism of methanol proceeds through the formation of formaldehyde, which is rapidly converted to formic acid or reacts with serum proteins and is not easily detected in the blood of methanol intoxicated patients or animals.

Method Principle (5-6)

The Catachem Methanol procedure is based on the affinity of an enzyme Alcohol Oxidase, from bacteria, to catalyze the oxidation of Methanol to Formaldehyde and H₂O₂, the Formaldehyde thus produced is subsequently converted to formic acid by the action of a second enzyme, Formaldehyde Dehydrogenase, in the presence of NAD. This two-point kinetic procedure is read at 340nm and the increase in absorbance is directly proportional to the concentration of Methanol in the serum sample.



Enzyme Reagent

Buffer	Each liter contains:
Alcohol Oxidase	≥4,000 Units
Formaldehyde Dehydrogenase	≥2,000 Units

NAD ≥1.80 mmol
Stabilizer and non-reactive ingredients

Enzyme Reagent Diluent

Surfactant
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 > 0.50 OD, 1cm 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 Methanol 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.

Materials Provided

Catachem Methanol Reagents
Catachem Methanol protein-based Calibrator and Controls with assigned values

Materials Required (Not Provided)

Spectrophotometer 340nm wavelength
Cuvettes 1cm light path
Timer to time incubation time
Pipette 0.5mL and 0.1mL for reagents
Pipette 0.006mL for sample

Calibration

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



Catachem, Inc., 353 Christian Street, Suite 2, Oxford, Connecticut, 06478 USA • Phone: (203) 262-0330 • catachem@catacheminc.com



NuuChem Diagnostics, LTD., 18 Killuney Road, Armagh, Northern Ireland, BT61 9HR • trevor.templeton@nuuchem.com



C604-0A

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

Methanol Reagent Kit
IVDD, MHRA, CE

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 Methanol Calibrator is utilized by the analyst to check calibration of a manual or automated Catachem Methanol 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

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

Directions for Use

Catachem's Methanol method requires a single reagent.

Procedure Limitations

Samples with Methanol values greater than 150mg/dL should be diluted 1:2 with physiological saline and re-assayed. Multiply results obtained by 2 to adjust for the sample dilution.

Analytical Parameters

Wavelength	340 nm
Temperature	37°C
Pathlength	1cm
Reaction Mode	End point
Reaction Time	5-6 minutes
Reaction Volume (R1)	0.5000mL
Sample Volume	0.0025mL
Total Volume	0.5025mL
Sample-to-reagent ratio	1:200

Assay Procedure

1. Bring the Methanol Working Reagent to room temperature.

2. Set the spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing deionized water.
3. Pipette 0.5mL of Working 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. Read the cuvettes reagent absorbance.
6. Pipette 0.0025mL of calibrator, controls and sample(s) into their respective cuvettes. Mix all cuvettes well.
7. Note the absorbance after 40 seconds (A1) and again after 150 seconds (A2).
8. Note Delta Absorbance absorbances.
9. Calculate the Methanol concentration (mg/dL) in the sample(s), as shown in calculations and results section.

Calculations and Results

$$\text{Methanol} \left(\frac{\text{mg}}{\text{dL}} \right) = \frac{\text{Sample} (A2 - A1)}{\text{Calibrator} (A2 - A1)} * \text{Calibrator} \left(\frac{\text{mg}}{\text{dL}} \right)$$

Example:

	<u>A1</u>	<u>A2</u>
Sample	0.20	0.30
Calibrator	0.20	0.50

$$\text{Calibrator} = 100 \text{ mg/dL}$$

$$\text{Methanol} \frac{\text{mg}}{\text{dL}} = \frac{0.3 - 0.2}{0.5 - 0.2} * 100 \frac{\text{mg}}{\text{dL}} = 33.3 \frac{\text{mg}}{\text{dL}}$$

Reference Values (5)

None detected ≤ 5 mg/dL
Potentially Toxic >25 mg/dL

Interfering Substances (5)

The following substances have no significant effect on the accuracy of this Methanol procedure at the concentrations stated.

- Hemoglobin ≤ 1,000mg/dL
- Triglycerides ≤ 1,000mg/dL
- Bilirubin ≤ 30 mg/dL
- Isopropanol ≤900mg/dL
- Ethylene Glycol ≤ 300mg/dL
- Ethanol ≤ 500mg/dL

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).



Catachem, Inc., 353 Christian Street, Suite 2, Oxford, Connecticut, 06478 USA • Phone: (203) 262-0330 • catachem@catacheminc.com

NuuChem Diagnostics, LTD., 18 Killuney Road, Armagh, Northern Ireland, BT61 9HR • trevor.templeton@nuuchem.com



C604-0A

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

Methanol Reagent Kit
IVDD, MHRA, CE

Method Performance Characteristics

Linearity: This procedure is linear over a range of 0-150mg/dL (0-8.33 mmol/L).

Limit of Detection: 2.7 mg/dL (as determined on a Beckman AU 480)

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

Sensitivity: Using a pathlength of 1cm, a Δ -absorbance of 0.01-0.015 per mg/dL should be obtained.

Precision: Precision data was obtained using three levels of protein-based controls and following the NCCLS EP5-T2 procedure (7).

The following results were observed:

Methanol Mean mg/dL	Within-Run Precision		Total Precision	
	SD mg/dL	CV %	SD mg/dL	CV %
24.35	0.468	1.929	0.641	2.632
49.31	1.025	2.097	1.525	2.709
93.83	1.284	1.357	1.346	1.428

NOTE: 1mg/dL = 0.312mM/L

Bibliography

1. Eelss JT. Formaldehyde poisoning, rapid metabolism to formic acid. J. Am Med Assoc 1981; 246:1237-8
2. Heck HA, Casanova-Schmitz M, Dodd PB, et al. Formaldehyde (CH₂O) concentrations in blood of humans and Ficher-344 rats exposed to (CH₂O) under controlled conditions. Am Ind Hyg Assoc J 1985; 46:1-3.
3. Miceli JN, Misiak PM. Air Formaldehyde and its association with body burdens [Abstract]. Clin Chem 1986; 32:1090.
4. McMartin KE, Martin-Amat G, Noker PE, et al. Lack of a role for formaldehyde in methanol poisoning in the monkey. Biochem Pharmacol 1979;28-645-9.
5. Vinet Bernard. Enzymatic Assay for the Specific Determination of Methanol in Serum. Clin Chem 1987; 22:23204-2208.
6. Wu AHB et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Recommendations for the use of Laboratory test to support Poisoned Patients Who Present to the Emergency Department. Clin Chem 2003; 49: 357-379
7. Evaluation of Precision Performance of Clinical Chemistry Devices. Second Edition. NCCLS Document EP5-T2. Vol. 12, No. 4.



Catachem, Inc., 353 Christian Street, Suite 2, Oxford, Connecticut, 06478 USA • Phone: (203) 262-0330 • catachem@catacheminc.com

NuuChem Diagnostics, LTD., 18 Killuney Road, Armagh, Northern Ireland, BT61 9HR • trevor.templeton@nuuchem.com