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Product Information

Methanol Assay Kit

Catalog Number **MAK353**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Methanol is the simplest alcohol, consisting only of a methyl group and a hydroxyl moiety. It is both an important industrial molecule (solvent, fuel, building block for chemical synthesis) and a biological metabolite; many bacteria generate methanol as a result of anaerobic metabolism. In humans, ingestion of large quantities of methanol is toxic and suppresses the nervous system; this is termed 'methanol poisoning'. As such, methanol is frequently used as an additive in industrial alcohols to prevent human consumption. The toxicity of methanol is primarily due to the biological product of its metabolism, formaldehyde, which is further metabolized into formic acid. At lower concentrations, methanol poisoning can cause loss of coordination and discomfort, and at higher concentrations will lead to kidney failure, blindness, and even death. In the gut, microbial breakdown of pectin-rich foods produces small amounts of methanol. In addition, some bacteria are capable of metabolizing methane gas, generating methanol, and so monitoring methanol concentration is also relevant for industrial purposes and renewable energy research.

The Methanol Assay Kit utilizes an enzymatic mechanism by which conversion of methanol is correlated stoichiometrically with generation of a colorimetric signal that can be quantified at 450 nm. The assay shows greater than 100-fold specificity for methanol over ethanol and can detect as little as 500 pmol of methanol.

The kit is suitable for the determination of methanol concentration in biological samples (serum, plasma, and urine), cell and tissue culture supernatants, fruit juice and other non-alcoholic beverages, and anaerobic microbial cultures.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Methanol Assay Buffer Catalog Number MAK353A	25 mL
Methanol Developer Catalog Number MAK353B	1 vial
Methanol Enzyme Mix Catalog Number MAK353C	1 vial
Methanol Probe Catalog Number MAK353D	1 vial
Pure Methanol Stock (24.7 M) Catalog Number MAK353E	500 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well flat-bottom plate. It is recommended to use clear plates for colorimetric assays
- Spectrophotometric multiwell plate reader, capable of 37 °C temperature setting
- Refrigerated microcentrifuge capable of RCF ≥10,000 rpm
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer

For R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

Methanol Assay Buffer – Warm to room temperature before use

Pure Methanol Stock (24.7 M) – Methanol is both flammable and toxic. Keep away from open flame, and avoid contact with skin and eyes.

Methanol Developer – Add 220 µL of Methanol Assay Buffer to the Developer. Mix well. Store at 4 °C. Use within one month once reconstituted. Do not freeze!

Enzyme Mix and Methanol Probe – Add 220 µL of Methanol Assay Buffer to each vial. Mix well. Store at –20 °C. Use within one month once reconstituted. *Do not combine these vials.*

Procedure

Note: **Extreme care should be taken to ensure that no alcohol vapors (ethanol, methanol, and propanol) are in the laboratory air where this assay is to be performed.** Alcohol vapors in the air will be rapidly absorbed by kit components resulting in very high background making the kit unusable. Laboratories where HPLC equipment and solvents are standing or where alcohol is used to wipe down laboratory benches or equipment are inappropriate locations to perform this assay.

Sample Preparation

Notes:

- Methanol concentration varies over a wide range depending on the sample. Methanol range concentrations in some biological samples are: human urine: 10–117 µmol methanol/mmol creatinine; human serum: 32–935 µM; human saliva: 1–142 µM. For unknown samples, it is suggested to perform a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.
- Metabolites found in biological samples interfere with the assay. If interference is observed in the diluted samples, prepare parallel sample wells(s) as sample background control(s) and make up volume to 50 µL with Methanol Assay Buffer.
- To ensure accurate determination of methanol in the test sample, or for samples having low concentrations of methanol, it is suggested to spike the samples with a known amount of methanol Standard (e.g., 4 nmol).

Biological fluid samples (plasma, serum, and urine)

Pretreat samples by spinning through a Corning Spin-X UF concentrator (10,000 rpm at 4 °C for 10 minutes) and use ultrafiltrate, discard the retentate

Anaerobic microbial culture

Samples should be pelleted (5,000 rpm at 4 °C for 10 minutes) and the supernatant medium filtered through a 10 kDa spin column before testing. Use the filtrate and discard the retentate.

Fruit juice and other non-alcoholic beverages

Samples may be filtered to enhance detection.

Sample Addition to Wells

Methanol, if present in the samples, will pass through into the filtrate. Pipette equal volumes (2–20) µL of each sample ultrafiltrate into two wells of a 96 well clear plate.

Standard Curve Preparation

Note: Methanol is a volatile liquid and small errors in the first dilution can alter the standards dramatically. Great care should be taken to ensure dilutions are prepared accurately. Ensure that additional methanol is not clinging to the sides of the pipette tip. Move pipette rapidly from methanol solution to the diluent to prevent loss of volume from the pipette tip.

1. Prepare a 1.235 M Methanol Solution by adding 50 µL of the 24.7 M Pure Methanol Stock to 950 µL of ultrapure water and mix.
2. Dilute the 1.235 M Methanol Solution to 50 mM by adding 10 µL to 237 µL of ultrapure water. Mix well.
3. Prepare a 1 mM Methanol Solution by adding 10 µL of 50 mM Methanol Solution to 490 µL Methanol Assay Buffer and mix.
4. Prepare Methanol Standards according to Table 1. Mix well.

Table 1.
Preparation of Methanol Standards

Well	1 mM Premix	Methanol Assay Buffer	Methanol (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mix

Mix enough reagent mix for the number of samples and standards to be performed. For each well (samples and standards), prepare 50 µL of Reaction Mix. For sample background wells, prepare 50 µL of Background Control Mix. Prepare according to Table 2.

Table 2.
Preparation of Reaction Mixes

Reagent	Reaction Mix	Background Control Mix
Methanol Assay Buffer	44 µL	46 µL
Methanol Probe	2 µL	2 µL
Methanol Enzyme Mix	2 µL	2 µL
Methanol Developer	2 µL	–

Add 50 µL Reaction Mix and 50 µL Background Control Mix to their respective sample wells.

Measurement

Incubate plate at 37 °C for 30 minutes and read absorbance at A_{450}.

Results

1. Subtract the 0 Methanol standard reading from all standard readings and plot the background-subtracted Methanol standards to generate the standard curve (from 0–10 nmol Methanol).
2. For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix. Apply the background-subtracted values to the standard curve to calculate methanol concentration:

Methanol (nmol/µL or mM) =

$$\frac{\text{Methanol from standard curve (nmol)}}{\text{Sample volume (µL)}} \times \text{Dilution Factor}$$

For spiked samples, correct for any sample interference by implementing following equation:

Methanol in spiked-sample well from standard curve =

$$\frac{A_{450}\text{ (corrected)} \times \text{MeOH spike (nmol)}}{(A_{450\text{sample}} + \text{MeOH Std (corrected)}) - (A_{450\text{sample}}\text{ (corrected)})}$$

Methanol Properties

MW = 32.04 g/mol
1 nmol Methanol = 32.04 ng
Density = 0.792 g/cm^3

Figure 1.
Typical Methanol curve

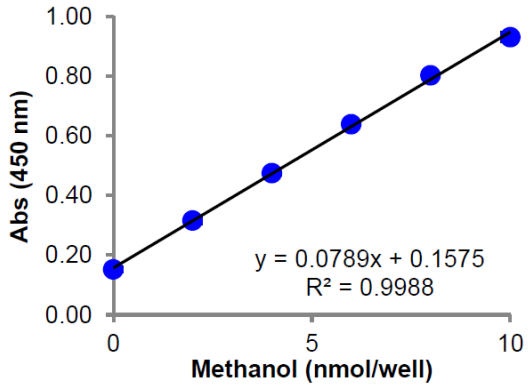
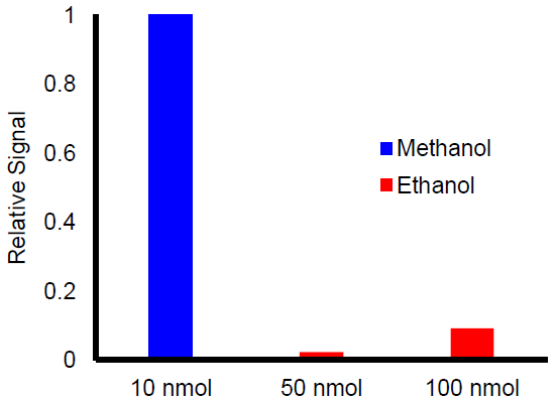
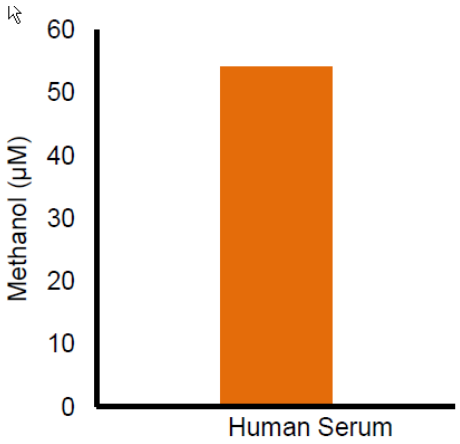


Figure 2.
Assay Selectivity



Response to various quantities of methanol and ethanol.

Figure 3.
Methanol in Serum



Methanol concentration was determined to be 54.4 µM in human serum (10 µL; undiluted).

Figure 4.
Methanol Recovery in Biological Fluids

	% Recovery	
Spiked Methanol (nmol)	Serum (10 µl)	Urine (5 µl)
2	109. 4	108. 8
4	101. 6	110. 5
6	100. 0	109. 9
8	94. 5	105. 8

Human serum (pooled) or urine was filtered and spiked with indicated quantities of methanol. Assays were run according to the procedure.

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