Technical Bulletin

Methanol Quantification Assay Kit

Sufficient for 200 fluorometric tests

CS0007

Product Description

Methanol is a simple alcohol, comprised of a hydroxyl group and a methyl group. It is typically used in antifreeze, fuel, and other products.[1] Methanol is found at very low levels in alcoholic beverages.[1] Small amounts of methanol are present in normal, healthy human individuals. However, higher levels of methanol are toxic. Methanol poisoning typically occurs following ingestion of a methanol-containing product.[2] The basis of the toxic effects of methanol is attributed to formate, to which methanol is eventually converted.[3,4] Elevated formate levels can lead to confusion, blindness, and death.[2]

The Methanol Quantification Assay Kit provides a simple and sensitive procedure for measuring methanol in various sample types. Methanol concentration is determined via a coupled enzymatic reaction. The fluorescence intensity, measured at λ_{ex} = 535 nm/λ_{em} = 590 nm, is proportional to the amount of methanol present in the sample.

The kit's linear range is 0.5-10 nmol.

This kit can be used to quantify methanol in biological samples such as serum and plasma, and in non-alcoholic and alcoholic beverages. The kit is highly specific to methanol, with less than 10% cross reactivity towards 100 nmol ethanol over 10 nmol methanol – effectively a specificity of over 100-fold.

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C, protected from light. The unopened kit is stable for 2 years as supplied.

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.

Components

This kit contains sufficient reagents for 200 fluorometric tests in 96-well plates.

Component	Component Number	Amount	Cap Color/ Container Information
Assay Buffer	CS0007A	50 mL	White cap/ bottle
Methanol Standard	CS0007B	1 mL	Red cap/vial
Enzyme I	CS0007C	100 µL	Yellow cap/ vial
Enzyme II	CS0007D	1 vial	Green cap/ vial
Developer	CS0007E	300 µL	Blue cap/vial
Probe	CS0007F	800 µL	Brown cap/ vial

Component Information

- Assay Buffer (CS0007A): Ready-to-use. Upon thawing, store at 2-8 °C. Equilibrate to room temperature before use.
- Methanol Standard (CS0007B): Pure 100% methanol (24.7 M). Upon thawing, store at 2-8 °C, protected from light. Equilibrate to room temperature before use.
- Enzyme I (CS0007C): Ready-to-use. Store at –20 °C. To avoid freeze/thaw cycles, it is recommended to prepare aliquots, and store the aliquots at –20 °C, protected from light. Keep on ice while in use.
- Enzyme II (CS0007D): Reconstitute with 600 µL of cold Assay Buffer. Mix gently by pipetting. To avoid freeze/thaw cycles, it is recommended to prepare aliquots, and store the aliquots at –20 °C, protected from light. Keep on ice while in use.

- Developer (CS0007E): Ready-to-use. To avoid freeze/thaw cycles, it is recommended to prepare aliquots, and store the aliquots at –20 °C, protected from light. Keep on ice while in use.
- Probe (CS0007F): Ready-to-use. Store at –20 °C, protected from light. Keep on ice while in use.

Equipment Required, But Not Provided

- 96-well black flat-bottom plates
- Fluorescence (λ_{ex} = 535 nm/λ_{em} = 590 nm) plate reader
- Fume Hood

Procedure

General Notes

- All samples and standards should be run in duplicate.
- A fresh set of standards should be prepared for each set of assays.
- Briefly centrifuge vials before opening.
- To avoid high background, it is important to keep the kit components and working solutions away from alcohol vapors. The use of a fume hood is recommended. When using a fume hood, remove any alcohol-containing vessels from the hood.
- **For convenience, an Excel-based calculation sheet is available on the Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Sample Preparation

- All assays (samples, standards, and blank) require 50 µL of sample for each reaction (well). Therefore, bring the sample to a final volume of 50 µL.
- When required, samples should be diluted in Assay Buffer. In case of high dilutions (≥100-fold), the initial dilutions can be performed in ultrapure water, whereas the final dilutions should be performed in Assay Buffer. For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.
- Beverages can be assayed directly or diluted if required.
- Serum and plasma (heparinized) samples should be deproteinated, by filtering through a 10 kDa spin column at 10,000 × *g* for 15 minutes at 4 °C. Discard the retentate and use the filtrate for the assay.

Methanol standard curve preparation:

1. Dilute the Methanol Standard (red cap vial) to a final concentration of 1 mM:
   1.1. Prepare an initial dilution: 50 µL of Methanol Standard with 950 µL of ultrapure water.
   1.2. Prepare a second dilution: 10 µL of the initial dilution with 237 µL of ultrapure water.
   1.3. Prepare a 1 mM methanol standard: 10 µL of the second dilution with 490 µL of Assay Buffer.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM methanol standard into a 96-well plate, to generate 0 (Blank), 2, 4, 6, 8, and 10 nmol/well standards, respectively.
3. Complete the volume to 50 µL with Assay buffer (see Table 1):

Table 1. Preparation of Methanol Standards*

Volume of 1 mM Methanol Standard	Assay Buffer Volume	Final Methanol Amount per Well
0 µL	50 µL	0 nmol (blank)
2 µL	48 µL	2 nmol
4 µL	46 µL	4 nmol
6 µL	44 µL	6 nmol
8 µL	42 µL	8 nmol
10 µL	40 µL	10 nmol

* Work in duplicate

Reaction Mix

Set up the Reaction Mix according to Table 2. 50 µL of Reaction Mix is required for each reaction (well). Multiply the volumes in Table 2 according to the number of wells in the assay. Mix gently by pipetting. Protect the Reaction Mix from light.

Table 2. Reaction Mix, per one well

Reagent	Cap color	Volume
Assay buffer	White	41.6 µL
Enzyme I	Yellow	0.5 µL
Enzyme II	Green	2.5 µL
Developer	Blue	1.4 µL
Probe	Brown	4 µL

Assay reaction

1. Add 50 µL of Reaction Mix to each of the standard and sample wells.
2. Mix well by pipetting.
3. Incubate the reaction for 15 minutes at 25 °C. Incubation up to 30 °C is possible. Protect the plate from light during the incubation.
4. Measure fluorescence intensity at λ_{ex} = 535 nm/λ_{em} = 590 nm.

Results

Calculations

- An Excel-based calculation sheet is available at the Product Detail Page. **Use this sheet to calculate the test results**.
- If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

1. Subtract the blank value (0 standard) from all standard and sample values.
2. Plot the fluorescence measured for each standard against the standard amount per well.
3. Determine the linear regression equation. Use it to calculate the methanol concentration in the sample:

[(Sample)/(Sample volume)] × DF = mM methanol

where:

Sample = Amount of methanol in unknown sample (nmol), calculated from the standard curve

Sample volume = Sample volume added into the wells (50 µL)

DF = Sample dilution factor (if the sample is not diluted, then the DF value is 1)

4. The molecular mass of methanol is 32.04 g/mol. Therefore, to convert the concentration from mM to mg/mL, multiply the result (in mM) by 3.2×10^{-3} g/mmol.

Sample Calculation

- Sample = 6 nmol (from standard curve)
- Sample volume = 50 µL
- DF = 100
- Concentration of methanol in the sample:

[(6 nmol)/(50 µL)] × 100 = 12 mmol/L = 12 mM

- Conversion to mg/mL:

(12 mmol/L) × (3.2×10^{-2} g/mmol) = 0.4 g/L = 0.4 mg/mL

References

1. Paine, A.J., and Dayan, A.D., *Hum. Exp. Toxicol.*, **20(11)**, 563-568 (2001).
2. Mégarbane, B. *et al.*, *Intensive Care Med.*, **31(2)**, 189-195 (2005).
3. Kruse, J.A., *Intensive Care Med.*, **18(7)**, 391-397 (1992).
4. "Methanol toxicity. Agency for Toxic Substances and Disease Registry". *Am. Fam. Physician*, **47(1)**, 163-171 (1993).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

CS0007bul Rev 03/22 DK,DT,NA,LB,GCY DAA00006