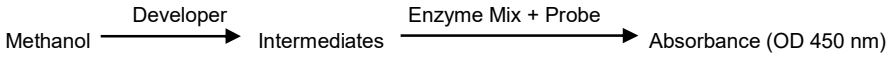
Methanol Assay Kit (Colorimetric) (#BN01072)

(Catalog # BN01072; 100 assays; Store at -20°C)

I. Introduction:

Methanol is the simplest alcohol, consisting exclusively 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. Assay Genie's 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. The method is suitable for use in a range of biological and consumable samples, and can detect as little as 500 pmol methanol.



II. Applications:

- Determination of methanol concentration in biological samples
- Determination of methanol levels in some agricultural and food products

III. Sample Type:

- Biological Samples: Serum, Plasma, Urine
- Fruit juice, other non-alcoholic beverages
- Microbial Cultures (anaerobic)

IV. Kit Contents:

Components	BN01072	Cap Code	Part Number
Methanol Assay Buffer	25 ml	WM	BN01072-1
Methanol Developer	1 vial	Purple	BN01072-2
Methanol Enzyme Mix	1 vial	Green	BN01072-3
Methanol Probe	1 vial	Red	BN01072-4
Pure Methanol Stock (24.7 M)	500 µl	Yellow	BN01072-5

V. User Supplied Reagents & Equipment:

- Plate Reader capable of 37°C setting and absorbance readings
- 96-well clear plates

VI. Storage and Reagents Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Methanol Assay Buffer:** Store at -20°C. Warm to RT before use. Stable for six months.
- **Pure Methanol Stock (100%):** Methanol is both flammable and toxic. Store at -20°C. 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. **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. *Do not combine these vials.*

VII. Methanol Determination Assay Protocol:

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.

1. **Sample Preparation:** For serum, plasma, and urine: pretreat samples by spinning through a 10 kDa spin column (10k rpm, 4 °C,10 min) and use ultrafiltrate. For microbial culture: samples should be pelleted (5k rpm, 4°C, 10 min) and the supernatant media filtered through a 10 kDa spin column before testing. Methanol, if present in the samples, will pass through into the filtrate. Pipet equal volume (2-20) µl of each sample ultrafiltrate into two wells of a 96-well clear plate. For beverages: samples may also be filtered to enhance detection. For all samples, keep the filtrate, and discard the retentate.

   Notes:

   a. 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, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

b. Metabolites found in biological samples interfere with the assay. If interference is observed in the diluted samples, prepare parallel samples (wells(s) as sample background control(s) and make up volume to 50 µl with Methanol Assay Buffer.

c. To ensure accurate determination of Methanol in the test sample or for samples having low concentrations of Methanol, we recommend spiking samples with a known amount of Methanol Standard (e.g. 4 nmol).

2. Standard Curve Preparation: Prepare fresh Methanol Standard as follows:

a. First, add 50 µl of of the 24.7 M Stock to 950 µL ddH_2O and mix. This will result in a 1.235 M Methanol Solution.

b. Dilute the 1.235 M Methanol Solution to 50 mM by adding 10 µl to 237 µl ddH_2O. Mix well.

c. To generate the 1 mM Methanol Stock, add 10 µl 50 mM Methanol Solution to 490 µl Methanol Assay Buffer and mix.

Add 0, 2, 4, 6, 8, and 10 µl of the Working Methanol Standard to each well individually to generate standards of 0, 2, 4, 6, 8, and 10 nmol Methanol/well. Adjust the volume of each well to 50 µl with Assay Buffer.

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 pipet tip. Move pipet rapidly from methanol solution to the diluent to prevent loss of volume from the pipet tip.

3. Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well (samples and standards), prepare 50 µl Reaction Mix. For sample background wells, prepare 50 µl Background Control Mix:

	Reaction Mix (per well)	Background Control Mix (per well)
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.

4. Measurement: Incubate plate at 30°C for 30 minutes and read absorbance at 450 nm.

5. Calculations: 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). 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:

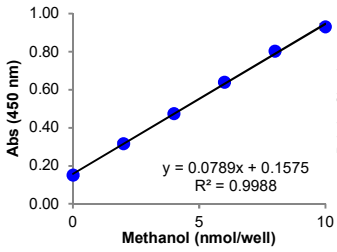
$$\textbf{Methanol Concentration} = \left(\frac{\text{Methanol amount from standard curve (nmol)}}{\text{vol. of sample(ml)}}\right) x\ Dilution\ Factor\ D\ \left(\frac{nmol}{\mu l}\ or\ mM\right)$$

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

$$\textbf{Methanol amount in spiked-sample well from standard curve} = \left(\frac{\text{ODsample(corrected)}}{\text{ODsample+Me}\quad \text{Std(corrected)}-(\text{ODsample (corrected)})}\right) \text{x MeOH spike (nmol)}$$

Methanol MW = 32. 04 g/mol; 1 nmol Methanol = 32. 04 ng; Density = 0. 792 g/cm^3

a)



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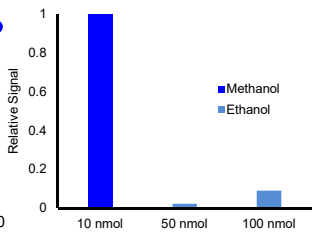
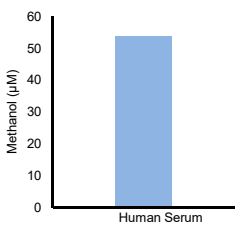


c)



d)

	% 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

Figure 1: (a) Methanol Standard Curve. (b) Selectivity of Assay: Response to various quantities of methanol and ethanol. **(c) Methanol in serum.** Methanol concentration was determined to be 54. 4 µM in human serum (10 µl; undiluted). **(d) Methanol Recovery in biological fluids:** Human serum (pooled) or urine was filtered and spiked with indicated quantities of methanol. Assays were run according to protocol.

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