Methanol Quantitation Assay (Colorimetric/ Fluorometric) Catalog: TBS2018
(100 Assays, Store at -20°C)

One-step Sensitive Quantitation for Methanol in Serum, Plasma, Urine and Other bio-samples

DESCRIPTION

Methanol (CH_3OH) is a simple alcohol that serves both as a biological metabolite and a toxic compound in humans and animals. Quantitative determination of methanol is very useful for life science and winery.

Tribo™ Methanol Assay Kit provides a rapid, simple, reproducible, and sensitive tool for methanol quantitation in plasma, serum, urine, and other samples. The methanol assay uses coupling enzyme reactions to produce color and fluorescence. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at λex/em = 530/590 nm is directly proportional to methanol concentration in the sample. The fluorometric assay is more sensitive than colorimetric assay.

Synonyms: methyl alcohol, wood alcohol.

APPLICATIONS

Direct Assays: Methanol concentration in serum, plasma, urine, and other bio-samples.

KEY FEATURES

Flexible: Suitable for colorimetric and fluorometric methods.
Accurate: Use 50 µL samples. Detection ranges 4 µM-1 mM in 96-well plate for colorimetric assay and 0.1µM-1 mM for fluorometric assay.
Simple and high-throughput: One-step procedure: just load-incubate-Read. Kits can be used as a robust method.

KIT CONTENTS

Component	Part Size
Assay Buffer	10 mL
Red Probe	0.12 mL
Methanol Standard (24.7M)	0.1 mL
Enzyme 1	0.12 mL
Enzyme 2	0.12 mL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 1 year. Protect from light.
Shipment with blue ice. Avoid frozen and thaw circles.

FLOROMETRIC PROTOCOL

Ensure the Reagent is at room temperature before use. Keep samples and enzyme on ice before the assay. It is recommended that all standards and samples be duplicated in the assay.

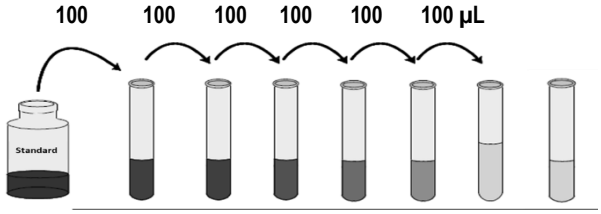
Sample Preparations:

Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample (1/2, ¼, or 1/8). Solid samples, such as tissues, can be first homogenized and extracted with PBS (tissues/Ethanol ratio of 1:8) for 1 h at 4°C, followed by centrifugation at 10,000g for 10min. The clear supernatants then can be measured as described for liquid samples. Add 50µL test samples directly into 96-well black plate.

Standard Curve Preparations:

1. Prepare 1.235 M Methanol Solution by adding 10 µL of 24.7 M Methanol Stock to 190 µL of ultrapure water and mix.
2. Prepare 50 mM Methanol Solution by adding 10 µL of 1.235 M to 237 µL of ultrapure water. Mix well.
3. Prepare 1 mM Methanol Solution by adding 10 µL of 50 mM Methanol Solution to 490 µL Methanol Assay Buffer and mix.
4. Label 1.5mL tube from Std1 to 8. As below the diagram. 100 µM Methanol Standard by take 30 µl of 1 mM methanol + 270 µL assay buffer solution as Std1 (100 µM).
5. Add 200uL of 1x Assay Buffer to Std2 to 8.
6. Make 3x series dilution from Std2 through 7 by transferring 100µL higher concentration of the standard solution to the next one. Std8 is 1x Assay Buffer alone as a standard 0 and Blank. The standard concentration range is 100, 33.3, 11.1, 3.7, 1.23, 0.41, 0.14 & 0 µM or 5, 1.6, 0.55, 0.185, 0.0617, 0.0205, 0.00685, 0 nmol/well.

Fig.1: Diagram of Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	270	200	200	200	200	200	200	200
Addition	1 mM	Std1	Std2	Std3	Std4	Std5	Std6	
Addition (µL)	30	100	100	100	100	100	100	
Final Conc (µM)	100	33.3	11.1	3.7	1.23	0.41	0.14	0

Work Solution

Mix 47 µL Assay Buffer with of 1 µL Enzyme 1, 1 µL Enzyme 2, and 1µL probe for 50 µL of each well.

Assay Procedures

1. Add 50 µL of standard or sample to each well of a black microplate in duplicate manner *(Note: the black microplate is for fluorescence detection)*.
2. Add 50 µL work solution to each well containing the Standard and test samples. Tap plate lightly to mix.
3. Incubate at 37°C for 30 minutes, protect from light.
4. Measure the fluorescence intensity using a microplate reader, equipped for excitation of 530nm and emission detection at 590 nm. *(Note: Because the assay is continuous (not terminated), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions).*

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COLORIMETRIC PROCEDURE

The colorimetric assay is similar with the fluorometric assay. Prepare the standards using fluorometric assay **Fig.1** as reference, e.g. 300 µM methanol =121 µL of 1 mM + 279 µL of assay buffer as Std1, then add 200uL of 1x Assay Buffer to Std2 to 8. Make 3x series dilution from Std2 through 7 by transferring 100µL higher concentration of the standard solution to the next one. Std8 is 1x Assay Buffer alone as a standard 0 and **Blank**. The standard concentration range is 300, 100, 33.3, 11.1, 3.7, 1.23, 0.412.5 & 0 µM or 15, 5, 1.6, 0.55, 0.185, 0.0617, 0.0205, 0 nmol/well.

1. Transfer 50 µL standards, samples into separate wells of a 96-well plate.
2. Add 50 µL Working Reagent (see fluorometric Procedure), tap plate to mix. Incubate 30-60 min at 37°C.
3. Read OD value at 570 nm (550-585 nm).

Calculation

Subtract the blank value (0 µM Standard) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the ethanol concentration of the Sample using the equation obtained from the linear regression of the standard curve.

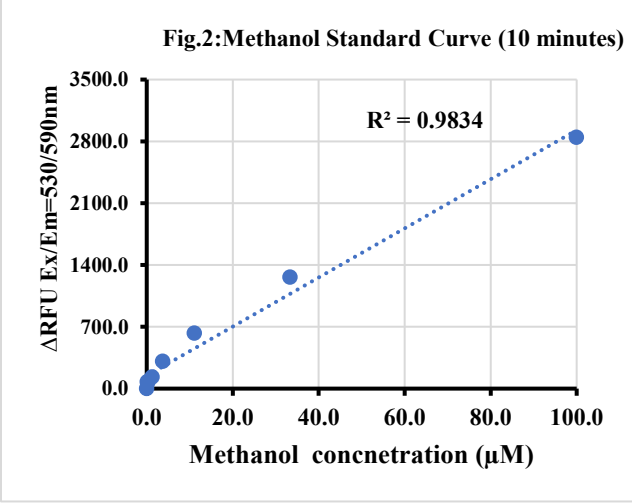
Methanol = N x (R_{sample}- R_{blank})/Slope (µM)

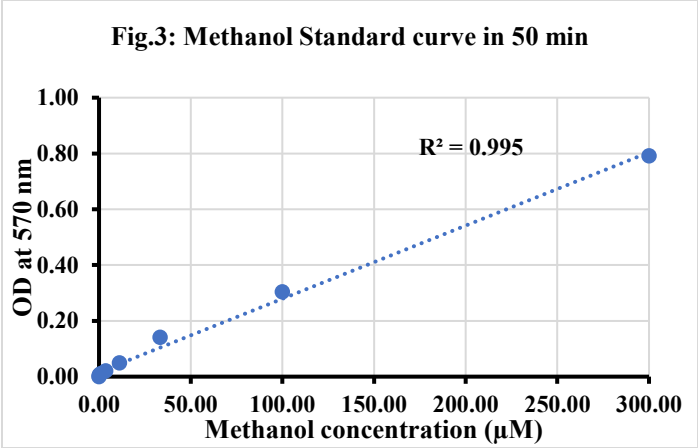
Where: R sample and R blank are optical density or fluorescence intensity readings of the sample and blank, respectively. N is the sample dilution factor.

Note1: If unknown sample results are over standard curve range, dilute samples with assay buffer, and repeat the assay.

TYPICAL DATA

The typical data is provided for only demonstration reference Shown in Fig.2 for fluorometric assay and Fig.3 for colorimetric assay.





RELATED PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
CCK-8 Cell Viability Assay (TBS2022)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Caspase-3 Colorimetric Assay kit (TBS2030)
AHCY Activity Fluorometric Assay (TBS2056)
Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102)
AHCY Inhibitor Screening Fluorometric Assay (TBS2099)

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