

Amplite® Methanol Quantification Assay Kit

 Catalog number: 40002
 Unit size: 100 Tests

Component	Storage	Amount (Cat No. 40002)
Component A: Amplite® Methanol Reagent	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: Assay Buffer	Freeze (< -15 °C), Minimize light exposure	1 Bottle (30 mL)
Component C: Methanol Assay Enzyme	Freeze (< -15 °C)	1 Vial
Component D: Methanol Standard	Freeze (< -15 °C)	1 Vial (200 µL)
Component E: DMSO	Refrigerated (2-8 °C)	1 Vial (100 µL)

OVERVIEW

The Amplite® Methanol Quantification Assay Kit provides a sensitive and reliable method for quantifying methanol in various sample types, including serum and plasma, and in beverages. Methanol (CH₃OH), the simplest alcohol, is widely used as a solvent, fuel additive, antifreeze component, and precursor in chemical synthesis. It is also produced by certain anaerobic microorganisms and is present at low levels in alcoholic beverages and in healthy individuals. Elevated concentrations are highly toxic, with poisoning resulting from ingestion of methanol-containing products. Toxicity is primarily due to its metabolic conversion to formate, which can accumulate and cause metabolic acidosis, neurological impairment, blindness, and death. The Amplite® Methanol Quantification Assay Kit quantifies methanol through a coupled enzymatic reaction, with the reaction product detected fluorometrically. Fluorescence intensity, measured at Ex/Em = 410/525 nm, is directly proportional to methanol concentration. The assay exhibits high specificity for methanol, with minimal cross-reactivity toward ethanol.

AT A GLANCE
Protocol Summary

1. Prepare test samples, methanol standards (50 µL).
2. Add methanol working solution (50 µL).
3. Incubate at RT for 30-60 minutes.
4. Measure the fluorescence at Ex/Em = 410/525, cut off = 495 nm.

Important Note

Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS
Fluorescence microplate reader

Cutoff	495 nm
Emission	525 nm
Excitation	410 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Amplite® Methanol Reagent Stock Solution (500X)

1. Prepare the 500X Amplite® methanol reagent stock solution by adding 20 µL of DMSO (Component E) to the vial containing Amplite® methanol reagent (Component A).

Methanol Standard Solution (1%)

1. Prepare a 1% methanol standard solution by adding 5 µL of 100% methanol standard (Component D) to 495 µL of methanol assay buffer (Component B). Mix thoroughly.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
<https://www.aatbio.com/tools/serial-dilution/40002>

Methanol Standard

Prepare the 0.1% methanol standard (STD7) by adding 50 µL of 1% methanol standard solution to 450 µL of methanol assay buffer (Component B). From STD7, take 150 µL and perform a 3-fold serial dilution in methanol assay buffer (Component B) to generate standards STD6 through STD1.

PREPARATION OF WORKING SOLUTION

1. Prepare the methanol working solution by adding 10 µL of the 500X Amplite® methanol reagent stock solution to 5 mL of the assay buffer (Component B) followed by addition of 10 µL of methanol assay enzyme (Component C). Mix thoroughly.

Note: 5 mL of the methanol working solution is enough for 1 plate. The working solution is unstable and should be used within 2 hours of preparation.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of methanol standards and test samples in a solid-backed 96-well microplate. STD = Methanol Standards (STD1-STD7, 0.000137% to 0.1%), BL = Blank Control, TS = Test Samples.

BL	BL	TS	TS
STD 1	STD 1
STD 2	STD 2
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
STD 1 - STD 7	50 µL	Serial Dilutions (0.000137 to 0.1%)
BL	50 µL	Methanol Assay Buffer
TS	50 µL	Test Sample

1. Prepare methanol standards (STD1–STD7), blank controls (BL), and test samples (TS) following the layouts specified in Tables 1 and 2. When using a 384-well plate, dispense 25 μL of reagent per well; for all other plate formats, dispense 50 μL per well.
2. Add 50 μL of the methanol working solution to each well designated for blank controls, methanol standards, and test samples. For assays performed in a 384-well plate format, add 25 μL of the working solution per well instead.
3. Incubate at room temperature for 30 to 60 minutes.
4. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 410/525 nm (Cutoff = 495nm).

EXAMPLE DATA ANALYSIS AND FIGURES

Fluorescence measurements from blank wells were used as background controls and subtracted from the fluorescence values obtained for the methanol standards and test samples. The resulting methanol standard curve and the specificity of the assay are presented in Figure 1.

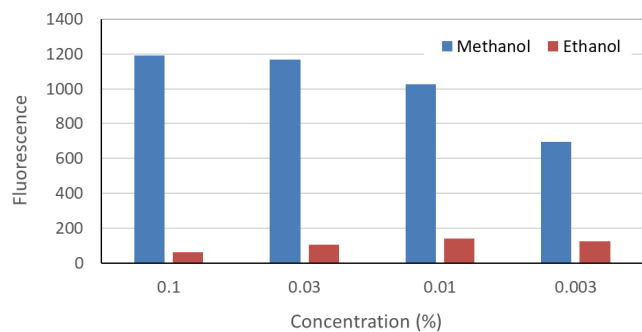
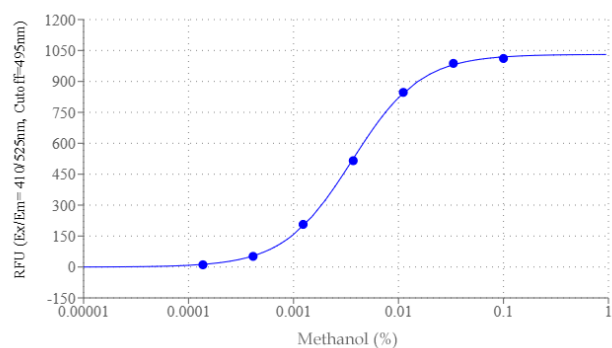


Figure 1. Methanol dose response curve (top) and specificity (bottom) measured with Amplite® Methanol Quantitation Kit on a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.