Amplite® Colorimetric Glucose Quantitation Kit

Catalog number: 40004
Unit size: 500 Tests

Component	Storage	Amount (Cat No. 40004)
Component A: Amplite® Red (light sensitive)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)
Component C: Horseradish Peroxidase (HRP)	Freeze (< -15 °C), Minimize light exposure	1 vial (10 units)
Component D: Glucose Oxidase	Freeze (< -15 °C), Minimize light exposure	1 vial (100 units)
Component E: DMSO	Freeze (< -15 °C)	1 vial (200 µL)
Component F: Glucose	Freeze (< -15 °C), Minimize light exposure	1 vial (144 mg)

OVERVIEW

Glucose, a monosaccharide, is the most important carbohydrate in biology. It is a source of energy and metabolic intermediate for cell growth. As one of the main products of photosynthesis, glucose starts cellular respiration in both prokaryotes and eukaryotes. Glucose level is a key diagnostic parameter for many metabolic disorders, e.g., diabetes. This Amplite® Colorimetric Glucose Quantitation Kit provides a quick and sensitive method for the measurement of glucose. It uses glucose oxidase-based enzyme coupled reactions to detect glucose through the production of hydrogen peroxide, which is monitored by our Amplite® Red peroxidase substrate. Amplite® Red peroxidase substrate can be read by an absorbance microplate reader at ~570 nm. The assay is robust, and can be readily adapted for a wide variety of applications that require the measurement of glucose. The assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. With the Amplite® Colorimetric Glucose Quantitation Kit, we can detect as little as 3 µM D-glucose.

AT A GLANCE

Protocol Summary

1. Prepare Glucose standards and/or test samples (50 µL)
2. Add Glucose Assay working solution (50 µL)
3. Incubate at 37°C for 10 - 30 minutes
4. Monitor absorbance increase at OD 570±5 nm

Important Note

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570 nm
Recommended plate	White or Black wall/Clear bottom

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® Red stock solution (250X)

Add 100 µL of DMSO (Component E) into the vial of Amplite® Red substrate (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20 ^{O}C.

Note: Avoid repeated freeze-thaw cycles. Note: The Amplite® Red substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Amplite® Red substrate is also unstable at high pH (> 8.5). Therefore, the reaction should be performed at pH 7–8. The provided assay buffer (pH 7.4) is recommended.

Horseradish Peroxidase (HRP) stock solution (10 U/mL)

Add 1 mL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).

Note: The unused HRP solution should be divided into single use aliquotes and stored at -20 ^{O}C.

Glucose Oxidase solution (100 U/mL)

Add 1 mL of Assay Buffer (Component B) into the vial of Glucose Oxidase (Component D).

Note: The unused Glucose Oxidase solution should be divided into single use aliquotes and stored at -20 ^{O}C.

Glucose stock solution (800mM)

Add 1 mL of Assay Buffer (Component B) into the vial of Glucose (Component F).

Note: The unused Glucose solution should be divided into single use aliquotes and stored at -20 ^{O}C.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40004

Glucose standard

Prepare a glucose standard by diluting the appropriate amount of the 800 mM glucose stock solution into assay buffer (Component B) to produce glucose concentrations of 100 µM. Then perform 1:2 serial dilutions in assay buffer (Component B) to get approximately 50, 25, 12.5, 6.3, 3.1 and 1.6 µM serially diluted glucose standards. A non-glucose buffer control is included as blank control.

PREPARATION OF WORKING SOLUTION

Table 1. Assay working solution for one clear bottom 96-well microplate (2X)

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Components	Volume
Amplite&trade Red Stock Solution (250x)	20 µL
HRP Stock Solution (10 U/mL)	100 µL
Glucose Oxidase Solution (100 U/mL)	100 µL
Assay Buffer	4.78 mL
Total volume	**5 mL**

SAMPLE EXPERIMENTAL PROTOCOL

Table 2. Layout of Glucose standards and test samples in a clear bottom 96-well microplate. GS = Glucose standard (GS1-GS7); BL = blank control; TS = test sample.

BL	BL	TS	TS
GS1	GS1	...	...
GS2	GS2	...	...
GS3	GS3		
GS4	GS4		
GS5	GS5		
GS6	GS6		
GS7	GS7		

Table 3. Reagent composition for each well

Well	Volume	Reagent
GS1 - GS7	50 µL	Serial Dilutions (1.6 -100 µM)
BL	50 µL	Assay Buffer (Compound B)
TS	50 µL	Test Sample

Glucose assay

1. Add glucose standards and glucose containing test samples into a 96-well clear bottom microplate as described in Tables 2 and 3.
2. Add 50 µL of Assay working solution into each well of glucose standard, blank control, and test samples (Table 2) to make the total glucose assay volume of 100 µL/well.

   Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.
3. Incubate the reaction for 10 to 30 minutes at 37 ^{O}C, protected from light.
4. Monitor the absorbance increase with an absorbance plate reader at OD = 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (OD (570 nm)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Glucose samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

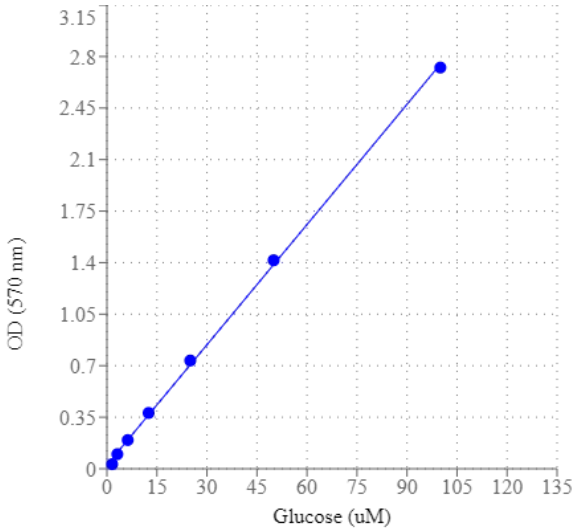


Figure 1. Glucose dose response was measured with Amplite® Colorimetric Glucose Quantitation Kit (Cat #40004) on a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices) with path check on. As low as 3 uM glucose was detected with 30 minutes incubation (n=3).

DISCLAIMER

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