Amplite™ Fluorimetric Glucose Quantitation Kit

Catalog number: 40005
Unit size: 500 Tests

Component	Storage	Amount
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. Glucose is one of the main products of photosynthesis and starts cellular respiration in both prokaryotes and eukaryotes. Glucose level is a key diagnostic parameter for many metabolic disorders. This glucose assay kit provides a quick and sensitive method for the measurement of glucose in various biological samples (e.g., serum, plasma, body fluid, food, growth medium, etc.). The kit uses our Amplite™Red substrate that making the kit recordable in a dual more, either fluorimetric or colorimetric readout. The kit provides all the essential components with an optimized assay protocol. The assay is robust, and can be readily adapted for high-throughput assays in a wide variety of applications that require the measurement of glucose. For example, the assay might be suitable for monitoring glucose level during fermentation and glucose feeding in protein expression processes. It might also be used for monitoring glucose transporters.

AT A GLANCE

Protocol summary

1. Prepare and add Glucose standards and/or test samples (50 µL)
2. Prepare and add Glucose Assay working solution (50 µL)
3. Incubate at 37°C for 10 - 30 minutes
4. Monitor fluroscence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 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.

1. 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 °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.

2. 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 °C.

3. 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 °C.

4. 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 °C.

PREPARATION OF STANDARD SOLUTION

glucose standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40005

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 30 µM. Then perform 1:3 serial dilutions in Assay Buffer (Component B) to get approximately 10, 3, 1, 0.3, 0.1 and 0.03 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)

Components	**Volume**
Amplite™ 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 solid black 96-well microplate. GS = Glucose standard (GS1-GS7); BL = blank control; TS = test sample.

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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

Glucose Standard	Blank Control	Test Sample
Serial Dilutions: 50 µL	Assay Buffer (Compound B): 50 µL	50 µL

Note High Concentration of glucose (e.g. 100 µM in test sample or standard) may cause reduced fluoroscence signal due to the overoxidation of Amplite™ red substrate (to a non-fluorescent product).

Glucose assay

1. Add glucose standards and glucose containing test samples into a 96-well solid black microplate as described in Tables 2 and 3.

2. Add 50 µL of Glucose 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 fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) 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

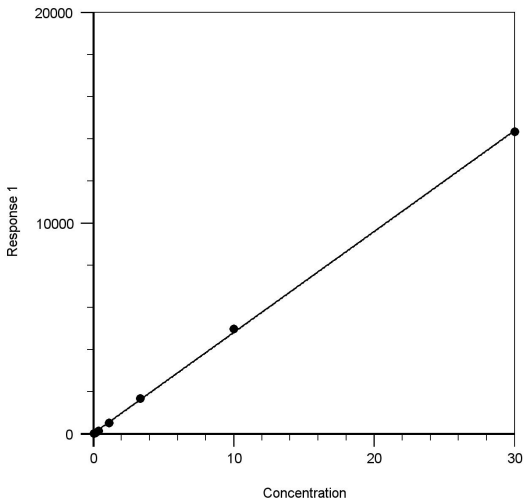

Figure 1. Glucose dose response was measured with Amplite™ Fluorimetric Glucose Quantitation Kit on a 96-well black plate using a Gemini microplate reader (Molecular Devices).

DISCLAIMER