Amplite™ Glucose Quantitation Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 40005 (500 assays)	Keep at -20 °C and protect from light	Fluorescence microplate readers

Introduction

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™ glucose assay 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 recorded in a dual mode, the fluorescent signal can be easily read by a fluorescence microplate reader at Ex/Em = 540/590 nm, or its absorption 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. It has demonstrated high sensitivity and low interference with excitation at 570 nm and emission at 590 nm. With the Amplite™ fluorimetric Glucose Quantitation Kit, we can detect as little as 3 µM D-glucose.

Kit Key Features

Sensitive:	Detect as low as 3 uM D-glucose in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Amplite™ Red (light-sensitive)	1 vial
Component B: Assay Buffer	1 bottle (50 mL)
Component C: Horseradish Peroxidase (HRP)	1 vial (10 units)
Component D: Glucose Oxidase	1 vial (100 units)
Component E: DMSO	1 vial (200 µL)
Component F: Glucose	1 vial (144 mg)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare assay reaction mixture (50 µL) → Add Glucose standards or test samples (50 µL) → Incubate at 37 °C for 10-30 minutes → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Note: Thaw all the kit components to room temperature before starting the experiment.

1. Prepare stock solutions:

1.1 250X Amplite™ Red stock solution: 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 1: Avoid repeated freeze-thaw cycles.

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Note 2: 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.

1.2 10 U/mL HRP stock solution: 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 aliquots and stored at -20°C.

1.3 100 U/mL glucose oxidase solution: 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 aliquots and stored at -20°C.

1.4 800 mM glucose stock solution: Add 1 mL of assay buffer (Component B) into the vial of glucose (Component F).
Note: The unused glucose solution should be stored at -20°C.

2. Prepare assay reaction mixture:

Prepare Assay reaction mixture according to the following tables, protected from light.

Table 1 Assay reaction mixture for one 96-well plate (2X)

Components	**Volume**
250X Amplite™ Red Stock Solution (from Step 1.1)	20 uL
10 U/mL HRP Stock Solution (from Step 1.2)	100 uL
100 U/mL Glucose Oxidase Solution (from Step 1.3)	100 uL
Assay Buffer (Component B)	4.78 mL
Total volume	5 mL

Table 2 Layout of glucose standards and test samples in a solid black 96-well microplate

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

Note: GS= Glucose standards, BL=Blank control, TS=test samples.

Table 3. Reagent composition for each well

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

**Note 1: Add the serially diluted glucose standards from 3 μM to 200 μM into each well from GS1 to GS7 in duplicate.*

Note 2: High concentration of glucose (e.g., 500 μM, final concentration) may cause reduced fluorescence signal due to the overoxidation of Amplite™ red substrate (to a non-fluorescent product).

3. Run Glucose assay:

3.1 Prepare a glucose standard by diluting the appropriate amount of the 800 mM glucose stock solution (from Step 1.4) into assay buffer (Component B) to produce glucose concentrations of 0 to 200 µM, each in a volume of 50 µL. A non-glucose buffer control is included as blank control. The final glucose concentrations should be twofold lower (i.e., 0 to 100 µM).

3.2 Add 50 µL of assay reaction mixture (from Step 2) into each well of glucose standard, blank control, and test samples (see Step 2, Table 3) 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.

3.3 Incubate the reaction for 10 to 30 minutes at 37 °C, protected from light.

3.4 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 nm).
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with glucose reactions. A glucose standard curve is shown in Figure 1.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

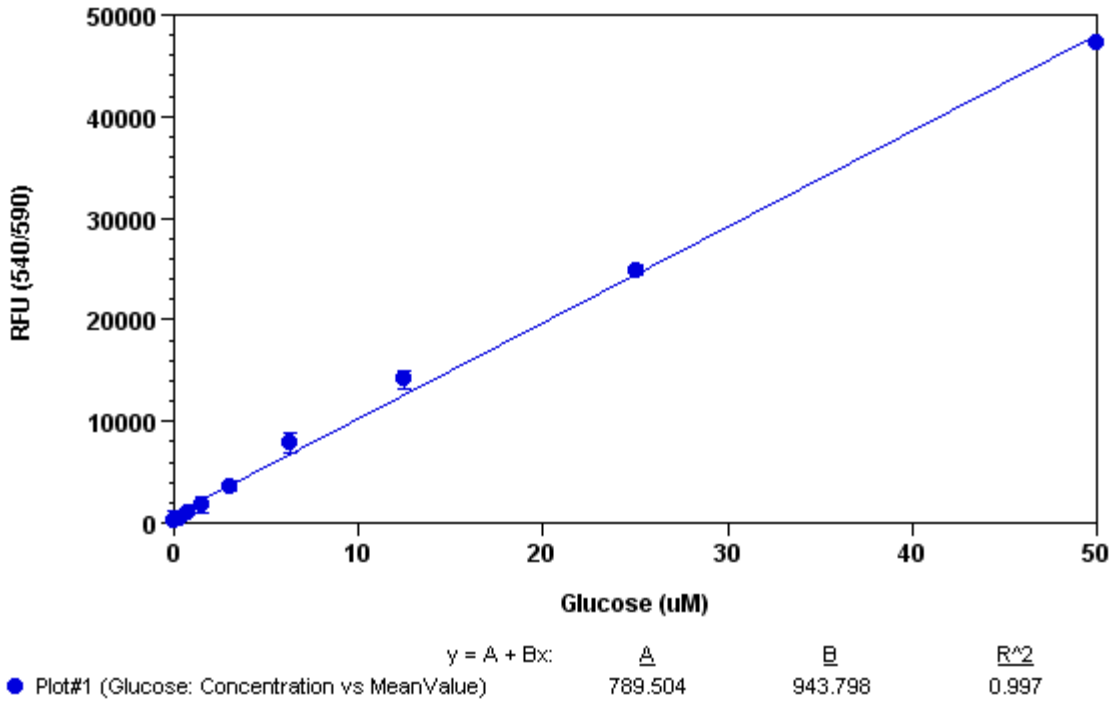

Figure 1. Glucose dose response was measured with Amplite™ Fluorimetric Glucose Quantitation Kit on a 96-well black plate using a Novostar microplate reader (BMG Labtech). As low as 3 μM glucose was detected with 30 minutes incubation (n=3).

References

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