Amplite™ Glucose Quantitation Kit **Red Fluorescence**

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #40005 (500 assays)	Keep in -20°C and avoid 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. 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 Amplite™ 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 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. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with 570 nm excitation 590 nm emission. With the Amplite™ Glucose Quantitation Kit, we have detected as little as 3 μM D-glucose.

The Amplite™ Glucose Quantitation Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. The kit uses our Amplite™ HRP substrate that making the kit recordable in a dual mode, the fluorescent signal can be easily read by either fluorescence microplate reader with Ex/Em = 530 to 570 nm/590 to 600 nm (maximum Ex/Em = 540 nm/590 nm) or absorbance microplate reader at 576±5 nm.

Kit Key Features

Sensitive:	The kit detects as low as 3 uM D-glucose in solution.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Component	**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 min →→Read fluorescence at Ex 540 nm/Em 590 nm

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

1. Prepare stock solutions:

1.1 ~~Amplite™ Red stock solution (250X):~~ Add 100 ∝L of DMSO (Component E) into the vial of Amplite™ Red substrate (Component A). The stock solution should be used promptly. Any remaining solution need be aliquoted and refrozen at -20°C.

Note 1: Avoid repeated freeze-thaw cycles.
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. It is also unstable at high pH (>8.5). The reactions 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 as single use aliquots and stored at -20°C.

1.3 ~~100U/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 as single use aliquots and stored at -20°C

1.4 ~~400 mM glucose stock solution:~~ Add 2 mL of assay buffer (Component B) into the vial of glucose (Component F).
Note: The unused glucose solution can stored at -20°C.

2. Prepare assay reaction mixture:

2.1 Prepare Assay reaction mixture according to the following table and kept from light:

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

Components	**Volume**
Amplite™ Red stock solution (250X, from step 1.1)	20 uL
10 U/ml HRP (from step 1.2)	100 uL
100 U/ml glucose oxidase (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 ∝M to 200 ∝M into wells from GS1 to GS7 in duplicate.*
Note 2: High concentration of glucose (e. g., 500 ∝M, 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 400 mM glucose stock solution (prepared 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 no-glucose buffer control is included as blank control. The final glucose concentrations will be twofold lower (i.e., 0 to 100 μM).

3.2 Add 50 μL of assay reaction mixture (from Step 2) to each well of the glucose standard, blank control, and test samples (see Step 2, table 3) so that the total glucose assay volume is 100 μL/well
Note: For a 384-well plate, add 25 μL sample, 25 μL of assay reaction mixture *per well.*

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

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

4. Run 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. The typical data are shown in Figure 1 (glucose standard curve).
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.

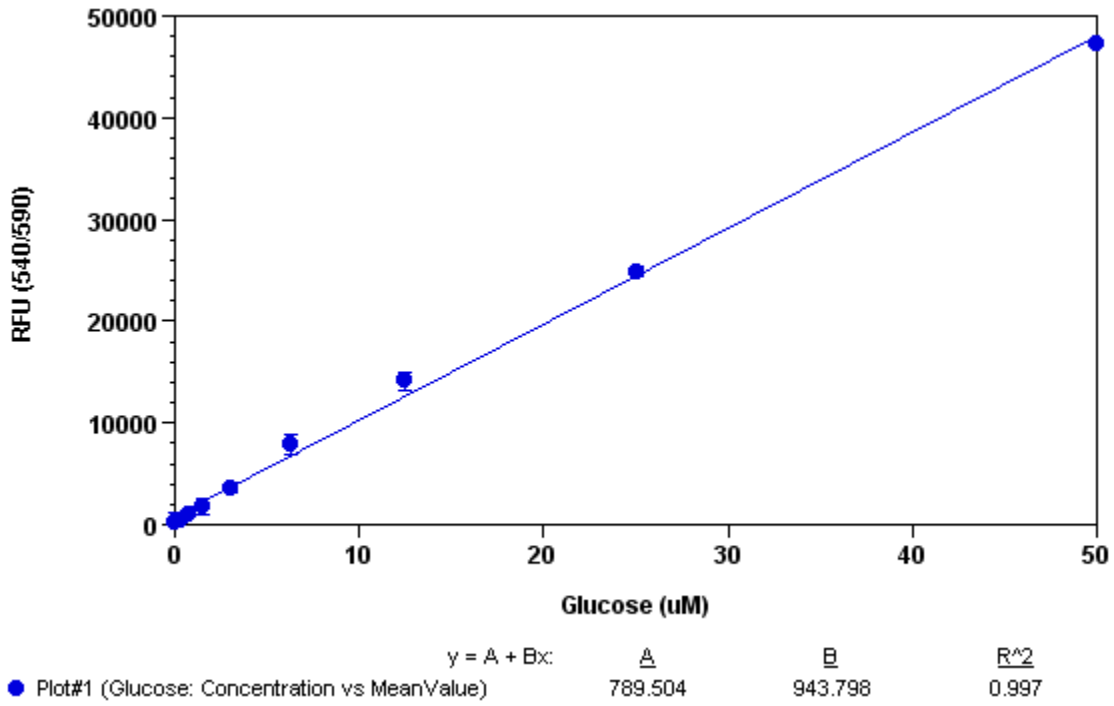

Figure 1. Glucose dose response on 96-well black plate using a Novostar microplate reader (BMG Labtech) measured with Amplite™ Glucose Quantitation Kit. As low as 3 ∝M of glucose can be detected with 30 minutes incubation time (n=3).

References:

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2. Delva P, Degan M, Pastori C, Faccini G, Lechi A. (2002) Glucose-induced alterations of intracellular ionized magnesium in human lymphocytes. Life Sci, 71, 2119.
3. Wang XT, Au SW, Lam VM, Engel PC. (2002) Recombinant human glucose-6-phosphate dehydrogenase. Evidence for a rapid-equilibrium random-order mechanism. Eur J Biochem, 269, 3417.
4. Leira F, Louzao MC, Vieites JM, Botana LM, Vieytes MR. (2002) Fluorescent microplate cell assay to measure uptake and metabolism of glucose in normal human lung fibroblasts. Toxicol In Vitro, 16, 267.