Amplite® Fluorimetric Glucose and Sucrose Assay Kit

Catalog number: 40010
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 40010)
Component A: Amplite® Red	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: Sucrose Assay Buffer	Freeze (< -15 °C), Minimize light exposure	25 mL
Component C: Invertase	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component D: Glucose Enzyme Mix	Freeze (< -15 °C), Minimize light exposure	1 Bottle
Component E: Sucrose Standard	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component F: DMSO	Freeze (< -15 °C)	1 Vial

OVERVIEW

Glucose, a key molecule in cellular energy metabolism, primarily functions during cellular respiration. It undergoes glycolytic breakdown for immediate ATP production or is converted into glycogen for energy storage. Sucrose, a disaccharide, is broken down into glucose and fructose by the enzyme invertase. The Amplite® Fluorimetric Glucose and Sucrose Assay Kit offers a convenient method for measuring glucose and sucrose levels in various biological samples, such as serum, plasma, body fluids, food, tissue extracts, and growth medium. This kit is useful for assessing aerobic glycolysis, pyruvate kinase activity, and metabolic profiles in different biological matrices. It is also employed for determining protein and sugar concentrations in food samples, as well as monitoring plasma insulin and glucose levels in murine models. To measure glucose levels, glucose oxidase specifically oxidizes free glucose, producing a fluorimetric product (Ex/Em = 571/584 nm) that is proportional to the glucose present. To measure sucrose levels, invertase is added to the reaction to convert sucrose into free glucose and fructose, allowing for the measurement of the total glucose level. The sucrose level is then calculated by subtracting the free glucose from the total glucose.

AT A GLANCE

Protocol Summary

1. Add sucrose standards or test samples (50 µL).
2. Add invertase working solution to each well (10 µL).
3. Incubate at 37°C for 30 minutes.
4. Add glucose working solution (40 µL) to each well.
5. Incubate at 37°C for 30 minutes, protected from light.
6. Monitor fluorescence at Ex/Em = 540/590 nm, cutoff 570 nm.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570
Emission	590
Excitation	540
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

Important Note: Before starting the experiment, thaw all of the kit components at room temperature.

Amplite® Red Stock Solution (100X)

1. Add 50 µL of DMSO (Component F) to the vial of Amplite® Red (Component A) to make a 100X Amplite® Red stock solution.

   Note: The stock solution should be used immediately. Any remaining solution should be made as single-use aliquots and stored at -20 °C. 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.

Invertase Stock Solution (200X)

1. Add 100 µL of Assay Buffer (Component B) to the vial of Invertase (Component C) to make a 200X invertase stock solution.

   Note: Any unused 200X invertase stock solution should be made as single-use aliquots and stored at -20 °C.

Sucrose Standard Solution (10 mM)

1. Add 1 mL of ddH2O to the vial of Sucrose Standard (Component E) to make a 10 mM sucrose standard solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40010

Sucrose Standard Dilution
Add 10 µL of 10 mM Sucrose stock solution into 990 µL of Assay Buffer (Component B) generate a 100 uM sucrose standard solution (STD7). Perform a 1:3 serial dilutions in Assay Buffer (Component B) to get serially diluted Sucrose standards (STD7 to STD1).

PREPARATION OF WORKING SOLUTION

Invertase Working Solution

1. To make an invertase working solution, add 50 µL of 200X invertase stock solution to 950 µL of Assay Buffer (Component B). Prepare the amount of working solution as needed.

Glucose Working Solution

1. Add 5 mL of Assay Buffer (Component B) to the bottle of Glucose

Enzyme Mix (Component D), and mix well.

2. Add 50 µL of 100X Amplite® Red stock solution to the bottle and mix well to create the Glucose working solution.

   Note: This working solution is enough for one 96-well plate. It is unstable at room temperature and should be used promptly within 2 hours and avoided exposure to light. Alternatively, one can make a 25X Glucose Enzyme Mixture stock solution by adding 200 µL of H2O to the bottle of Component A and then prepare the working solution by mixing the stock solution with assay buffer (Component B) and 100X Amplite Red stock solution proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. The layout of Sucrose standards and test samples in a clear bottom 96-well microplate. STD=Sucrose Standards (STD1-STD7, 0.14 to 100uM), BL=Blank Control, TS=Test Samples.

BL	BL	Positive Control	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 (0.14 to 100 µM)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	Test Sample

1. Prepare sucrose standards (STD), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For each test sample requiring sucrose detection, prepare 2 sets of replicates: one set for sucrose/total glucose detection, and one set for glucose detection.

2. Add 10 µL of invertase working solution to each of the sucrose standards and sucrose/total glucose detection wells. Add 10 µL of Assay Buffer (Component B) to the glucose detection wells.

3. Incubate the plate at 37°C for 30 minutes.

4. Add 40 µL of the glucose working solution to each of the wells. Mix well by using a horizontal shaker or by pipetting.

5. Incubate the plate at 37°C for 30 minutes, protected from light.

6. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 540/590 m, cutoff 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

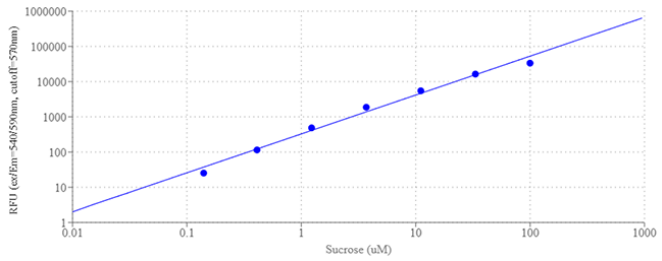

Figure 1. Sucrose dose response was measured with Amplite® Colorimetric Glucose and Sucrose Assay Kit on a 96-well clear bottom microplate after 30 minutes of incubation using a Gemini microplate reader (Molecular Devices) at Ex/Em = 540/590 nm, cutoff = 570.

APPENDIX

Calculating the Sucrose Concentrations

The standard curve of Sucrose is shown above in Figure 1. To calculate the sucrose concentrations of the samples according to the standard curve, 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

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