Amplite® Fluorimetric Glycogen Assay Kit *Red Fluorescence*

Catalog number: 40014
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 40014)
Component A: Amplite® Red	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Glycogen Hydrolysis Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component C: Glycogen Hydrolysis Enzyme	Freeze (< -15 °C)	1 vial
Component D: Glycogen Assay Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component E: Glycogen Assay Enzyme	Freeze (< -15 °C)	1 vial
Component F: Glycogen Standard (2mg/ml)	Freeze (< -15 °C)	1 vial (200 µL)
Component G: DMSO	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

The Amplite® Fluorimetric Glycogen Assay Kit provides a streamlined assay for the sensitive quantification of glycogen using a reliable two-step enzymatic process. In the first step, glycogen is enzymatically hydrolyzed into glucose. In the second step, the released glucose undergoes an oxidation reaction that produces hydrogen peroxide, which subsequently reacts with the proprietary Amplite® Red fluorogenic dye to generate a red fluorescent signal. The fluorescence intensity, which is directly proportional to the glycogen concentration, can be easily measured using a standard fluorescence microplate reader at Ex/Em of 540/590nm.

The kit is compatible with a wide range of biological samples, including cell lysates, tissue extracts, and serum samples. The assay protocol is easy to follow and produces a stable fluorescent signal, allowing glycogen measurements for high-throughput screening or routine analysis in metabolic research, diabetes studies, and other related applications.

AT A GLANCE

1. Prepare test samples along with serially diluted glycogen standards (40 µL).
2. Add glycogen hydrolysis enzyme working solution (10 µL).
3. Incubate at RT for 30 minutes.
4. Add glycogen assay working solution (50 µL).
5. Incubate at RT for 30 minutes.
6. Measure the fluorescence at Ex/Em = 540/590 nm.

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

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 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

Amplite® Red Stock Solution (100X)

Add 100 µL of DMSO (Component G) into Amplite® Red (Component A) to make 100X Amplite™ Red stock solution

Glycogen Hydrolysis Enzyme Stock Solution (20X)

Add 100 µL distilled water to Glycogen Hydrolysis Enzyme (Component C) to make a 20X Glycogen Hydrolysis Enzyme stock solution.

Glycogen Assay Enzyme Stock Solution (50X)

Add 100 µL distilled water to Glycogen Assay Enzyme (Component E) to make 50X Glycogen Assay Enzyme Stock Solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/40014

Glycogen Standard Dilution
Add 25 µL of 2 mg/mL Glycogen Standard solution (Component F) into 975 µL of Glycogen Hydrolysis Buffer (Component B) to get 50 µg/mL glycogen standard solution (STD7). Take 500 µL (STD7) and perform 2X serial dilutions in Glycogen Hydrolysis Buffer (Component B) to get 25, 12.5, 6.25, 3.125, 1.56, 0.781 µg/mL glycogen standard solutions (STD6 to STD1).

PREPARATION OF WORKING SOLUTION

Glycogen Hydrolysis Enzyme Working Solution:

Add 10 µL of 20X Glycogen Hydrolysis Enzyme stock solution to 190 µL of Glycogen Hydrolysis Buffer (Component B).

Note: 200 µL Glycogen Hydrolysis Enzyme working solution is for 20 assays with 96-well plates. Please prepare the volume as needed proportionally. The working solution is not stable, prepare it freshly, use promptly and avoid direct exposure to light.

Glycogen Assay Enzyme Working Solution:

Add 10 µL of 100X Amplite® Red stock solution and 20 µL of 50X Glycogen Assay Enzyme Stock Solution to 970 µL of Glycogen Assay Buffer (Component D).

Note: 1mL Glycogen Assay Enzyme Working Solution is for 20 assays with 96-well plates. Please prepare the volume as needed proportionally. The working solution is not stable, use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare Glycogen Standards (STD1-7), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 96-well plate, add 40 µL per well, and for a 384-well plate, use 20 µL per well instead of 40 µL.
2. Add 10 µL of Glycogen Hydrolysis Enzyme working solution to each

well of blank control, glycogen positive control and test samples. For a 384-well plate, add 5 µL working solution into each well instead.

3. Incubate at RT for 30 minutes.
4. Add 50 µL of Glycogen Assay Enzyme working solution to each well of blank control, glycogen positive control and test samples. For a 384-well plate, add 25 uL working solution into each well instead.
5. Incubate at RT for 30 minutes.
6. Measure fluorescence intensity at Ex/Em = 540/590 nm (cutoff = 570nm).

Table 1. Layout of glycogen standards and test samples in a clear bottom 96-well microplate. STD = glycogen standards (STD1-STD7, 0.6 to 40 µg/ml), BL = Blank Control, TS = Test Samples.

BL	BL	Positive Control	Cell content
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
STD1-STD7	40 µL	Serial Dilutions (0.6 to 40 µg/ml)
BL	40 µL	Hydrolysis buffer
TS	40 µL	Test Sample

EXAMPLE DATA ANALYSIS AND FIGURES

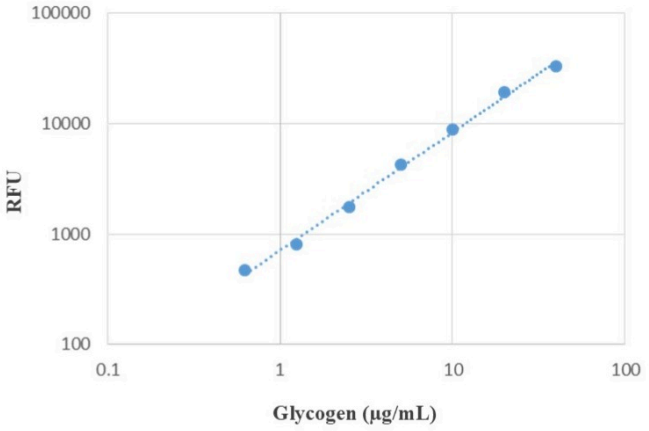

Figure 1. Glycogen dose response was measured with Amplite® Fluorimetric Glycogen Assay Kit (Cat. #40014) on a 96-well black solid microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em of 540/590 nm (Cutoff 570 nm).

DISCLAIMER

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