

Amplite® Fluorimetric Amyloglucosidase Assay Kit *Red Fluorescence*

Catalog number: 13817

Unit size: 100 Tests

Component	Storage	Amount (Cat No. 13817)
Component A: Amplite® Red	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Amyloglucosidase Reaction Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component C: Amyloglucosidase Substrate	Freeze (< -15 °C)	1 vial (200 µL)
Component D: Assay Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component E: Amyloglucosidase Detection Enzyme	Freeze (< -15 °C)	1 vial
Component F: Amyloglucosidase Standard	Freeze (< -15 °C)	1 vial (1U/vial)
Component G: DMSO	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

The Amplite® Fluorimetric Amyloglucosidase Assay Kit provides a streamlined and sensitive assay for detecting amyloglucosidase activity through a fluorescence-based approach. In this assay, amyloglucosidase catalyzes a reaction that produces a detectable product, which then undergoes a secondary reaction to generate a red fluorescent signal. The fluorescence intensity is directly proportional to the enzyme activity and can be easily measured using a standard fluorescence microplate reader with appropriate excitation and emission settings.

This kit is compatible with a wide range of enzyme sources and biological samples, making it ideal for enzyme characterization, inhibitor screening, and metabolic research. The assay protocol is simple to perform and yields a stable fluorescent signal, allowing for amyloglucosidase detection in high-throughput screening or routine amyloglucosidase activity analysis.

AT A GLANCE

1. Prepare test samples along with serially diluted Amyloglucosidase standards (10 µL).
2. Add Amyloglucosidase Substrate working solution (40 µL).
3. Incubate at RT for 30 minutes.
4. Add Amyloglucosidase Detection working solution (50 µL).
5. Incubate at RT for 30 minutes.
6. Measure the fluorescence intensity 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

Amyloglucosidase Detection Enzyme Stock Solution (50X)

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

Amyloglucosidase Stock Solution (10U/ml)

Add 100 µL distilled water to Amyloglucosidase Standard (Component F) to make a 10U/mL stock solution.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
<https://www.aatbio.com/tools/serial-dilution/13817>

Amyloglucosidase Standard Dilution

Add 15 µL of 10 U/mL Amyloglucosidase stock solution into 985 µL of Amyloglucosidase Reaction Buffer (Component B) to get 150 mU/mL Amyloglucosidase standard solution (STD7). Take 500 µL (STD7) and perform 2X serial dilutions in Amyloglucosidase Reaction Buffer (Component B) to get 75, 37.5, 18.8, 9.4, 4.68, 2.34 mU/mL Amyloglucosidase standard solutions (STD6 to STD1).

PREPARATION OF WORKING SOLUTION
Amyloglucosidase Substrate Working Solution:

Add 20 µL Amyloglucosidase Substrate (Component C) to 800 µL of Amyloglucosidase Reaction Buffer (Component B) to make Amyloglucosidase Substrate working solution.

Note: 800 µL Amyloglucosidase Substrate 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 and use promptly.

Amyloglucosidase Detection Working Solution:

Add 10 µL of 100X Amplite® Red stock solution, 20 µL of 50X Amyloglucosidase Detection Enzyme stock solution in 970 µL of Assay Buffer (Component D).

Note: 1mL Amyloglucosidase Detection Enzyme Stock 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 Amyloglucosidase 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 10 µL per well, and for a 384-well

plate, use 5 µL per well instead.

2. Add 40 µL of Amyloglucosidase Substrate working solution to each well of blank control, positive control and test samples. For a 384-well plate, add 20 µL working solution into each well instead.

Note: The final Amyloglucosidase concentration in each well are 30, 15, 7.5, 3.75, 1.88, 0.94, 0.468 mU/mL after adding Amyloglucosidase Substrate working solution.

3. Incubate at RT for 30 minutes.

4. Add 50 µL of Amyloglucosidase Detection working solution to each well of blank control, positive control and test samples. For a 384-well plate, add 25 µL 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).

DISCLAIMER

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Table 1. Layout of Amyloglucosidase standards and test samples in a clear bottom 96-well microplate. STD = Amyloglucosidase Standards (STD1-STD7, 2.34 to 150 mU/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 (2.34 to 150mU/mL)
BL	40 µL	Amyloglucosidase Reaction Buffer
TS	40 µL	Test Sample

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

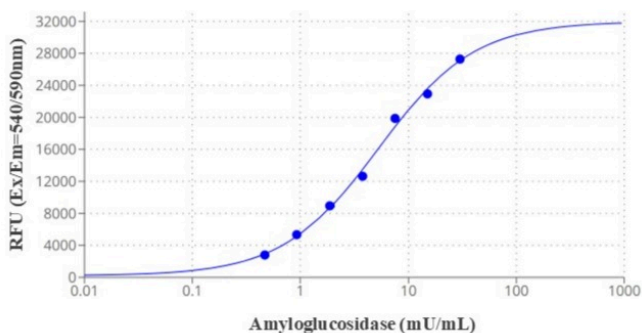


Figure 1. Amyloglucosidase dose response was measured with Amplite® Fluorimetric Amyloglucosidase Assay Kit (Cat. #13817) on a 96-well solid black microplate using a Gemini microplate reader (Molecular Devices) at Ex/Em = 540/590 nm (Cutoff 570 nm).