Amplite® Colorimetric Gama-Glutamyltransferase (GGT) Activity Assay Kit

Catalog number: 11801
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11801)
Component A: GGT Assay Buffer	Freeze (< -15 °C)	1 Bottle (25 mL)
Component B: GGT Substrate	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component C: GGT Positive Control	Freeze (< -15 °C), Minimize light exposure	1 Vial (50 µg)
Component D: pNA Standard	Freeze (< -15 °C)	1 Vial (0.4 mL)

OVERVIEW

The Amplite® Colorimetric Gamma-Glutamyltransferase (GGT) Activity Assay Kit provides an efficient and straightforward method for measuring GGT activity across various sample types. This assay utilizes a coupled enzyme reaction in which GGT catalyzes the conversion of a substrate into a colored product detectable at 418 nm, directly reflecting the GGT concentration in the sample. It is compatible with a wide range of biological samples, including cells, tissue extracts, and serum, and is suitable for high-throughput systems. γ-Glutamyltransferase (also known as GGT) is a mammalian enzyme playing an important role in the anti-oxidant defense mechanism of cells. It is located in cell membranes and is known for facilitating the transfer of γ-glutamyl moieties between molecules during glutathione regulation and xenobiotic detoxification. Elevated levels of serum GGT are linked to cardiovascular and liver diseases, metabolic syndrome, and oxidative stress. Consequently, GGT is a valuable biomarker for liver dysfunction and disorders related to oxidative stress.

AT A GLANCE

Protocol Summary

1. Prepare the test samples, GGT positive control, and the serially diluted pNA standards (50 µL).
2. Add the GGT working solution (50 µL).
3. Incubate for 10-30 minutes at 37 °C.
4. Measure the absorbance at 418 nm.

Important Note

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	418 nm
Recommended plate	Clear bottom

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

GGT Positive Control Stock Solution

1. Reconstitute the GGT Positive Control (Component F) with 100 µL of ddH2O to make a 500 µg/mL GGT Positive Control stock solution. Mix well by pipetting and store at -20 °C.

   Note: Must be used within 1 month of reconstitution. Avoid freeze/thaw cycles.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11801

pNA Standard

Add 50 µL of the 2 mM pNA standard solution to 450 µL of the GGT Assay Buffer (Component A) to make a 200 µM pNA standard solution (STD7). Then, take 250 µL of STD7 and perform a 2X serial dilutions with GGT Assay Buffer to create a series of diluted pNA standards (STD6 to STD1).

PREPARATION OF WORKING SOLUTION

GGT Working Solution

1. Reconstitute the GGT Substrate (Component B) by adding 5 mL of the GGT Assay Buffer (Component A). Mix thoroughly until well combined. Protect the mixture from light and store it at -20°C.

   Note: Avoid freeze/thaw cycles.

Test Samples

1. Tissue and cells can be homogenized using the GGT assay buffer. To remove any insoluble material, centrifuge the homogenized sample at 13,000xg for 10 minutes. Serum samples can be added directly to the wells without any prior treatment. Adjust the volume of all samples to 50 µL by adding GGT assay buffer.

SAMPLE EXPERIMENTAL PROTOCOL

GGT Positive Control

1. Prepare one or more GGT positive control samples along with the test sample. The recommended concentration is 20 µg/mL in GGT Assay Buffer. For example, for a 20 µg/mL positive control, add 20 µL of the GGT positive control stock solution to 480 µL of GGT Assay Buffer.

Table 1. Layout of pNA standards and test samples in a 96-well solid black microplate. (STD = pNA Standards (STD1-STD7, 3.125-200 µM), 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 (3.125 to 200 µM)
BL	50 µL	GGT Assay Buffer
GGT Positive Control	50 µL	GGT Positive Control
TS	50 µL	Test Sample

1. Prepare pNA standards (STD1-7), blank controls (BL), GGT Positive Control, and test samples (TS) as outlined in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of GGT Working Solution to each well containing the pNA standard, blank control, GGT Positive Control, and test samples. For a 384-well plate, add 25 µL of GGT Working Solution to each well instead.

3. Incubate at room temperature for 10-30 minutes, protected from light.

4. Monitor the absorbance intensity with an absorbance microplate reader at 418 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The absorbance reading from the blank wells (containing assay buffer only) is used as a control and subtracted from the readings of wells containing pNA standards, GGT positive controls, and test samples. Figure 1 shows the standard curve for pNA. To determine the GGT concentrations in your samples using this standard curve, we recommend using the Online Linear Regression Calculator available at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

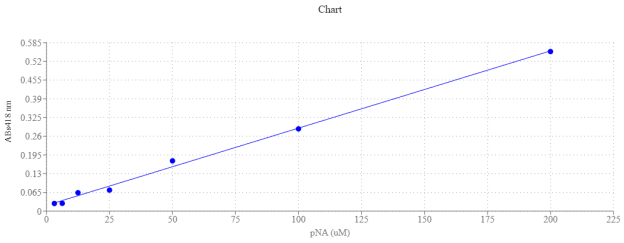


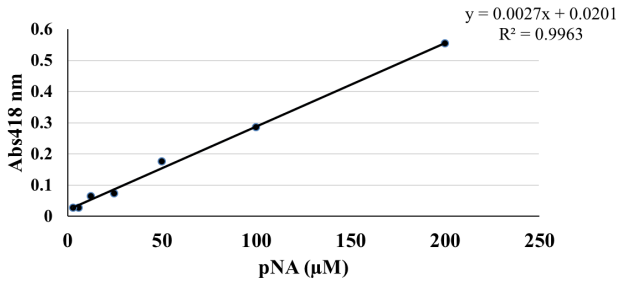
Figure 1. The pNA dose response was measured using the Amplite® Colorimetric Gama-Glutamyltransferase (GGT) Activity Assay Kit on a 96-well clear bottom microplate. The measurements were taken at 418 nm with a ClarioStar microplate reader (BMG).

Data Analysis

Example GGT Activity Calculation

- GGT positive control: 20 µg/mL

1) Plot pNA Calibration curve at 30 minutes



2) Calculate pNA generated during 30 minutes

GGT-Positive control (20 µg/mL) Abs418 nm	pNA generated in 30 min (µM)	µM/min	mU/mL
0.497	111.4	3.04	3.04

Note:

- 1 unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute
- nmole/min/mL=uM/min=mU/mL

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