Gamma-Glutamyl Transferase Assay Kit

Catalog Number KA7488

100 assays

Version: 01

Intended for research use only

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Table of Contents

Introduction 3

Background 3

General Information 4

Materials Supplied 4

Storage Instruction 4

Materials Required but Not Supplied 4

Precautions for Use 4

Assay Protocol 5

Reagent Preparation 5

Sample Preparation 5

Assay Procedure 5

Data Analysis 6

Calculation of Results 6

Performance Characteristics 6

Resources 7

References 7

Plate Layout 8

Introduction

Background

γ-GLUTAMYL TRANSFERASE (GGT, E.C. 2.3.2.2) is an enzyme that catalyzes the transfer of a glutamyl group to an acceptor, such as a peptide or amino acid. GGT plays a major role in the breakdown of glutathione and is found in many tissues, but is more commonly found in the liver. Elevated levels of GGT may indicate liver disease or damage to the bile ducts, and in clinical practice it is often tested along with other liver function tests. Gamma-Glutamyl Transferase Assay Kit provides a convenient colorimetric method to measure GGT activity. In this assay, GGT hydrolyzes a synthetic substrate to release p-nitroanilide (pNA), which absorbs at 405 nm. The increase in absorbance at 405 nm is directly proportional to the enzyme activity.

✓ Applications

For quantitative determination of gamma-glutamyl transferase enzyme activity in biological samples

✓ Key Features

- Safe and sensitive. Non-radioactive assay. Use as little as 10 µL of sample. Linear detection range in 96-well plate: 0.7 to 200 U/L activity.
- Fast and convenient. The procedure involves addition of a single ready-to-use reagent and incubation for 30 min. Room temperature assay. No 37°C incubator is needed.
- High-throughput. Homogeneous "mix-incubate-measure" type assay.
  Can be readily automated to assay thousands of samples per day.

General Information

Materials Supplied

List of component

Component	Amount
Reagent	10 mL
pNA Standard, 100 mM standard	50 µL

Storage Instruction

The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Materials Required but Not Supplied

- ✓ GGT enzyme as a positive control, If desired
- ✓ Pipetting devices
- ✓ Accessories (e.g. Multi-channel pipettor)
- ✓ Clear flat-bottom 96-well plates
- ✓ Centrifuge tubes
- ✓ Plate reader

Precautions for Use

- ✓ Reagents are for research use only.
- ✓ Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Reagent Preparation

✓ Standard Preparation

Prepare a 6000 µM Standard Premix by combining 6 µL of 100 mM pNA Standard and 94 µL of dH2O. Prepare all standards according to table below.

No	Premix + dH_2O	Total Volume	Std (µM)
1	50 µL + 0 µL	50 µL	6000
2	30 µL + 20 µL	50 µL	3600
3	15 µL + 35 µL	50 µL	1800
4	0 µL + 50 µL	50 µL	0

Standards: Transfer 10 µL of each Standard dilution to separate wells of a clear 96-well plate.

Sample Preparation

Serum and plasma can be assayed directly.

✓ Cell Lysate

Collect cells and wash 3x with PBS. Homogenize or sonicate cells in an appropriate volume of cold PBS. Centrifuge at 14,000 x g for 5 min. Remove supernatant for assay.

For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor n.

Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. This assay can be run at room temperature or 37°C. Prior to assay, equilibrate Reagent to desired temperature. Briefly centrifuge tubes before use.

1. Transfer 10 µL of each sample to separate wells.
2. To each Standard well (Standards #1 – 4), add 90 µL dH_2O. To each Sample well, add 90 µL Reagent. Tap plate briefly to mix.
3. Read OD_{405nm} immediately (OD_0) on a plate reader. Incubate protected from light at desired assay temperature for 30 min, then read OD_{405nm} again (OD_{30}). Alternatively, after step 2, record kinetics at OD_{405nm} at desired assay temperature for at least 30 min.

Data Analysis

Calculation of Results

Subtract the blank value (Standard #4) from the standard values and plot ΔF against the standard concentrations. Determine the slope (μM-1) and calculate the asparaginase activity in each Sample as follows:

$$\text{GGT Activity} = \frac{OD_{30} - OD_0}{\text{Slope } (\mu M^{-1}) \times t\,(\text{min})} \times n\ (\text{U/L})$$

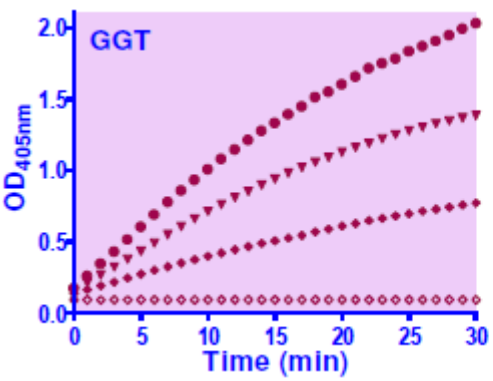
where OD_{30} and OD0 are the OD_{405nm} values at 30 min and 0 min, respectively. t is the reaction time (30 min). n is the dilution factor.

Unit definition: 1 Unit (U) of GGT will catalyze the release of 1 μmole of pNA per min at pH 8.0.

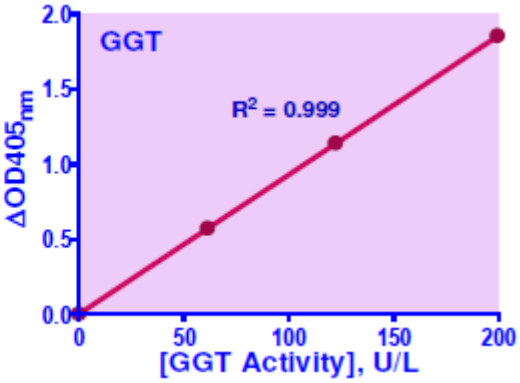
If sample GGT activity exceeds 200 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. If kinetics are recorded, any two time points in which the activity remains linear can be chosen for analysis. Use OD values from the latter time point to calculate the slope from the standards and adjust t to the chosen time interval.

Performance Characteristics

GGT activity was determined in triplicate following the protocol. The values were 9.4 ± 0.22 U/L for bovine serum, 6.5 ± 0.56 U/L for human serum, 0.77 ± 0.15 U/L for canine plasma, 7.3 ± 0.22 U/L for HEPG2 cell lysate (1.04 mg/mL protein). These values are examples and are not intended as expected values.



Raw Kinetic Data



GGT Activity (30min, 25°C)

Resources

References

1. Kunutsor, S. K. (2016). Gamma-glutamyltransferase-friend or foe within?. Liver international : official journal of the International Association for the Study of the Liver. 36(12):1723–1734.
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3. Hanigan, M. H. (1998). γ-Glutamyl transpeptidase, a glutathionase: Its expression and function in carcinogenesis. Chemico-Biological Interactions. 111-112:333–342.

Plate Layout

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