QuantiChrom™ γ-Glutamyl Transferase Assay Kit (DGGT-100)

Quantitative Colorimetric Assay for γ-Glutamyl Transferase Activity

DESCRIPTION

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. BioAssay Systems' DGGT-100 kit provides a convenient colorimetric method to measure GGT activity. In this assay, GGT hydrolyzes a synthetic substrate to release p-nitroanilide (*p*NA), which absorbs at 405 nm. The increase in absorbance at 405 nm is directly proportional to the enzyme activity.

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.

APPLICATIONS

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

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent: 10 mL

***p*NA Standard:** 50 µL 100 mM standard

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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.

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*.

Standard Preparation: Prepare a 6000 µM Standard Premix by combining 6 µL of 100 mM pNA Standard and 94 µL of dH_2O. Prepare all standards according to table below.

No.	Premix + dH_2O	Total Volume (µL)	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.

Reaction Preparation:

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.

CALCULATION

Using the OD values from 30 min, subtract the blank value (Standard #4) from the standard values and plot ΔOD against the standard concentrations. Determine the slope (μM^{-1}) of the *p*NA standard curve and calculate the GGT 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 OD_0 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.

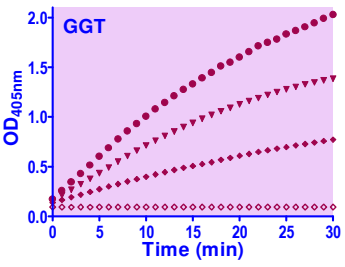
Note: If sample GGT activity exceeds 200 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. If kinetics are recorded, any two time points in which the activity remains linear can be chosen for analysis. Use OD values from the latter time point to calculate the slope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

MATERIALS REQUIRED, BUT NOT PROVIDED

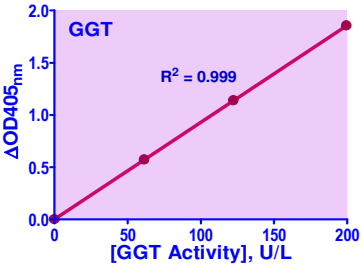
GGT enzyme (e.g. Lee Biosolutions, cat# 315-10) as a positive control, if desired. Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

EXAMPLES

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)

LITERATURE

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.

2. Brennan, P. N., Dillon, J. F., & Tapper, E. B. (2022). Gamma-Glutamyl Transferase (γ-GT) - an old dog with new tricks?. *Liver international : official journal of the International Association for the Study of the Liver*. 42(1):9–15.

3. Hanigan, M. H. (1998). γ-Glutamyl transpeptidase, a glutathionase: Its expression and function in carcinogenesis. Chemico-Biological Interactions. 111-112:333–342.