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Product Information

γ-Glutamyltransferase (GGT) Activity Colorimetric Assay Kit

Catalog Number **MAK089**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

γ-Glutamyltransferase (GGT, EC 2.3.2.2) is a membrane-bound protein that catalyzes the transfer of γ-glutamyl moieties to acceptor molecules such as amino acids or peptides. GGT plays a key role in the γ-glutamyl cycle, a critical pathway for glutathione homeostasis as well as the detoxification of xenobiotics. Serum GGT levels can be elevated in many pathophysiological conditions such as cardiovascular disease, chronic liver disease, and metabolic syndrome. Elevated serum levels of GGT can also be indicative of oxidative stress.

The GGT Activity Colorimetric Assay kit provides a simple and direct procedure for measuring GGT activity in a variety of samples. GGT activity is determined by a coupled enzyme assay, in which the GGT transfers the γ-glutamyl group from the substrate L-γ-Glutamyl-*p*-nitroanilide, liberating the chromogen *p*-nitroanilide (*p*NA, 418 nm) proportional to the GGT present. One unit of GGT is the amount of enzyme that will generate 1.0 μmole of *p*NA per minute at 37 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

GGT Assay Buffer Catalog Number MAK089A	25 mL
GGT Substrate Catalog Number MAK089B	1 ea
GGT Positive Control Catalog Number MAK089C	1 vl
*p*NA Standard, 2 mM Catalog Number MAK089D	0.4 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

GGT Assay Buffer – Allow buffer to come to room temperature before use.

GGT Substrate Solution – Reconstitute with 10 mL of GGT Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use because the GGT substrate solution is unstable at room temperature and can hydrolyze, increasing assay background.

GGT Positive Control – Reconstitute with 100 μL of water. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C. Use within 1 month of reconstitution.

2 mM *p*NA Standard – Just prior to use, warm for 1–2 minutes at 37 °C to thaw the DMSO solution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

*p*NA Standards for Colorimetric Detection
Add 0, 4, 8, 12, 16, and 20 µL of the 2 mM standard solution into a 96 well plate, generating 0 (blank), 8, 16, 24, 32, and 40 nmole/well standards. Add GGT Assay Buffer to each well to bring the volume to 100 µL.

Sample Preparation
Tissue (10 mg) or cells (1×10^6) can be homogenized in 200 µL of ice-cold GGT Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to the wells.

For the positive control, add 10 µL of the GGT positive control solution to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 10 µL with GGT Assay Buffer.

Assay Reaction
1. Add 90 µL of GGT Substrate Solution to each well containing test samples. Do not add to *p*NA Standards.

2. Incubate the plate at 37 °C. After 3 minutes, take the initial measurement ($T_{initial}$). Measure the absorbance at 418 nm at the initial time $(A_{418})_{initial}$.
   Note: It is essential $(A_{418})_{initial}$ is in the linear range of the standard curve.

3. Continue to incubate the plate at 37 °C taking measurements (A_{418}) every 5 minutes. Protect the plate from light during the incubation.

4. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (40 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

5. The final measurement $[(A_{418})_{final}]$ for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Plot the *p*NA standard curve from the initial measurement ($T_{initial}$).

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{418} = (A_{418})_{final} - (A_{418})_{initial}$$

Compare the ΔA_{418} of each sample to the standard curve to determine the amount of *p*NA generated between $T_{initial}$ and T_{final} (B).

The GGT activity of a sample may be determined by the following equation:

$$\text{GGT Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.
Reaction Time = $T_{final} - T_{initial}$ (minutes)
V = sample volume (mL) added to well

GGT activity is reported as nmole/min/mL = milliunit/mL
One unit of GGT is the amount of enzyme that will generate 1.0 μmole of *p*NA per minute at 37 °C.

Example:
*p*NA amount (B) = 25 nmole
First reading ($T_{initial}$) = 3 minute
Second reading (T_{final}) = 32 minutes
Sample volume (V) = 0.01 mL
Sample dilution is 1

GGT activity is:

$$\frac{25 \times 1}{(32-3) \times 0.01} = 86.2 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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