Gamma Glutamyl Transferase (GGT) Activity Colorimetric Assay Kit (#BN00997)

(Catalog #BN00997; 100 reactions; Store kit at –20°C)

I. Introduction:

The Gamma-Glutamyl Transferase (GGT; EC 2.3.2.2) is an enzyme that transfers gamma-glutamyl functional groups. It is found in many tissues, the most notable one being the liver, and has significance in medicine as a diagnostic marker. Assay Genie's Gamma-Glutamyl Transferase Assay Kit provides a convenient tool for sensitive detection of the GGT in a variety of samples. The GGT in sample will recognize L-γ-Glutamyl-*p*NA as a specific substrate leading to proportional color development. The activity of GGT can be easily quantified colorimetrically (λ = 418 nm). This assay detects GGT activity as low as 0.5 mIU.

II. Kit Contents:

Components	BN00997	Cap Code	Part Number
GGT Assay Buffer	25 ml	WM	BN00997-1
GGT Substrate	1 Bottle	NM	BN00997-2
GGT Positive Control	1 vial	Green	BN00997-3
*p*NA Standard (2mM)	400 µl	Yellow	BN00997-4

III. Storage and Handling:

Store the kit at -20°C, protected from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

GGT Substrate Solution: Add 10 ml assay buffer into substrate bottle and mix well. Take out enough substrate solution (90 µl per assay) for the number of assays to be performed in experiment. Store the rest of the GGT Substrate Solution into -20°C quickly. **Note:** The GGT Substrate solution is unstable at room temperature (can be hydrolyzed by itself) which increases the assay background.

GGT Positive Control: Reconstitute with 100 µl diH_2O. Pipette up and down several times to completely dissolve the pellet into solution (**Don't vortex**). Aliquot enough GGT Positive Control (10 µl per assay) for the number of assays to be performed in each experiment and aliquot and freeze the rest immediately at -20°C for future use. The GGT Positive Control is stable for up to 1 month at -20°C after reconstitution or freeze-thaw cycles (< 5 times). Keep the GGT Positive Control on ice during the preparation.

V. GGT Activity Assay Protocol:

1. ***p*NA Standard Curve: (Warm for 1-2 min at 37°C to completely melt DMSO):**
   Add 0, 4, 8, 12, 16, 20 µl of the 2 mM *p*NA standard solution into a 96-well plate in duplicate to generate 0, 8, 16, 24, 32, 40 nmol/well standard. Adjust the final volume to 100 µl with GGT Assay Buffer.

2. **Sample Preparations:**
   Tissues (10 mg) or cells (1×10^6) can be homogenized in the 200 µl GGT Assay Buffer then centrifuged (13,000 x g, 10 min.) to remove insoluble material. Serum samples (10 µl) can be directly added into each well. Prepare test samples to 10 µl/well with GGT Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the linear range of the standard curve.

3. **Reaction Mix:**
   Add 90 µl GGT Substrate Mix into each well containing the test samples and positive controls. Mix well. **Do not add to *p*NA Standards**.

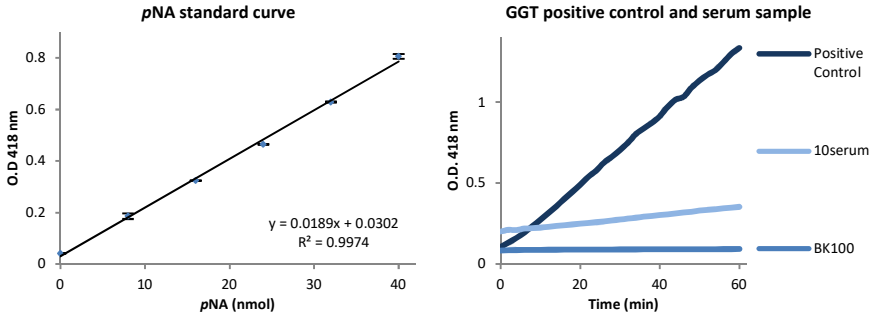
4. **Measurement:** For *p*NA Standard Curve, measure OD at 418 nm in a microplate reader. For the samples and positive controls, incubate the mix for 3 min at 37°C, then measure OD at 418 nm in a microplate reader (A_0), incubate for another 30 min to 2 hr at 37°C to measure OD at 418 nm again (A_1); incubation times will depend on the GGT activity in the samples. We recommend measuring the OD in a kinetic method (preferably every 3- 5 min) and choose the period of linear range which falls within pNA Standard Curve to calculate the GGT activity of the samples.

5. **Calculation:** Plot the *p*NA standard Curve, then calculate the GGT activity of the test samples: $\Delta OD = A_1 - A_0$, apply the ΔOD to the *p*NA standard curve to get B nmol of *p*NA generated by GGT in the given time.

$$\textbf{GGT Activity} = \frac{\mathbf{B}}{\mathbf{T}\times\mathbf{V}} \times \textbf{Sample Dilution Factor} = \textbf{nmol/min/ml} = \textbf{mU/ml}$$

Where: **B** is the *p*NA amount from standard Curve (in nmol)
T is the time incubated (in min)
V is the sample volume added into the reaction well (in ml)

Unit Definition: One unit GGT will generate 1.0 µmol of *p*NA per min at 37°C.
Note: One *p*NA unit ≈ 1.5 IU.



FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures 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	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• 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 tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		