



GGT Activity Assay Kit

Catalog # EA-7094

(For Research Use Only)

Introduction

Gamma-glutamyl transferase (GGT) is an important enzyme that is involved in amino acid transport and glutathione metabolism. This enzyme catalyzes the transfer of gamma-glutamyl groups between peptides and amino acids, which plays a key role in maintaining cellular antioxidant defenses. GGT is found primarily in the liver, kidneys, pancreas, and bile ducts. Because GGT is released into the bloodstream during liver or bile duct injury, it is a clinically significant biomarker used to assess liver function and detect hepatobiliary disease. Elevated GGT levels are commonly associated with conditions such as alcohol-related liver disease, bile duct obstruction, hepatitis, and pancreatic disorders.

Principle of the assay

The GGT assay kit utilizes a chromogenic substrate, γ -glutamyl-p-nitroanilide (GPNA), that releases a colored product when it hydrolyzed into pNA by GGT. The pNA product is yellow and can be measured spectrophotometrically at an absorbance of 405 nm.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 560 nm or fluorescence at 530nm/590nm

Materials Provided

- GPNA Substrate (-20°C)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x g for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

GGT Measurement

1. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
GPNA Substrate	1 μ L
PBS	49 μ L
Total	50 μ L

2. Add 50 μ L of reaction mix to each well of the plate.
3. Add 50 μ L of sample to each well with reaction mix and mix thoroughly.
4. Cover the plate and incubate at room temperature for 10 minutes away from light.
5. Measure the absorbance of the plate in a plate reader at 405 nm.