Signosis, Inc.
Innovative Plate Assay Solutions

Liver Enzyme Assay Combo Kit

Catalog # EA-7095 **(For Research Use Only)**

Introduction

Alananine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) are key enzymes measured in clinical chemistry to assess liver function and detect hepatobiliary or systemic disease. Together, these enzymes provide a complementary panel for evaluating liver damage patterns and distinguishing between hepatocellular and cholestatic disease. Signosis' Liver Enzyme Assay Combo Kit provides a comprehensive solution for measuring the activity of these key liver enzymes in a single, streamlined workflow.

Principle

ALT Assay

The ALT assay kit measures the activity of ALT by detecting its ability to transfer an amino group from L-alanine to alpha-ketoglutarate. This reaction produces pyruvate, which is quantified by another enzyme reaction. Pyruvate is oxidized by pyruvate oxidase to form a pyruvate byproduct and hydrogen peroxide. Then, the pyruvate level, which is proportional to ALT activity in the sample, is determined by quantifying the hydrogen peroxide generated by the enzyme reaction with a fluorogenic probe that can be measured with a spectrophotometer.

AST Assay

The AST assay kit measures the activity of AST by detecting its ability to transfer an amino group from L-aspartate to alpha-ketoglutarate. This reaction produces glutamate, which is quantified by another enzyme reaction. Glutamate is oxidized by glutamate oxidase to form a glutamate byproduct and hydrogen peroxide. Then, the glutamate level, which is proportional to AST activity in the sample, is determined by quantifying the hydrogen peroxide generated by the enzyme reaction with a fluorogenic probe that can be measured with a spectrophotometer.

ALP Assay

The ALP assay kit utilizes a chromogenic substrate, p-nitrophenyl phosphate (pNPP), that releases a colored product when it hydrolyzed into pNP by ALP. Under alkaline conditions, pNP becomes yellow and can be measured spectrophotometrically at an absorbance of 405 nm.

GGT 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

- Alanine Reagent (-20ºC)
- Aspartate Reagent (-20ºC)
- Ketoglutarate Reagent (-20ºC)
- 1x GLOX Enzyme Stock (-80ºC)
- Probe Reagent (-20ºC)
- HRP Reagent (4ºC)
- pNPP Substrate (-20ºC)
- GPNA Substrate (-20ºC)

****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.

ALT 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)
Alanine Reagent	0.05 µL
Ketoglutarate Reagent	0.05 µL
1x POX Enzyme	0.5 µL
PBS	48.95 µL
Total	50 µL

2. Any unused enzyme stock can be stored at -80°C for future use.
3. Add 50 µL of reaction mix to each well of the plate.
4. Add 5 µL of sample or standard to each well with reaction mix and mix thoroughly.
5. Cover the plate and incubate at room temperature for 30 minutes.
6. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

Component	Detection Mix (per well/sample)
Probe Reagent	0.5 µL
HRP Reagent	1 µL
PBS	48.5 µL
Total	50 µL

7. Add 50 µL of detection mix to each reaction well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
8. Cover the plate and incubate at room temperature away from light for 30-60 minutes.
   Exposure to light will produce background signal in wells
9. For a stronger signal, the plate can be incubated for another 1-2 hours away from light.
10. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.

AST 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)
Aspartate Reagent	0.05 µL
Ketoglutarate Reagent	0.05 µL
1x GLOX Enzyme	0.5 µL
PBS	48.95 µL
Total	50 µL

2. Any unused enzyme stock can be stored at -80°C for future use.
3. Add 50 µL of reaction mix to each well of the plate.
4. Add 5 µL of sample or standard to each well with reaction mix and mix thoroughly.
5. Cover the plate and incubate at room temperature for 30 minutes.
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10. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.

ALP 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)
pNPP Substrate	1 µL
PBS	49 µL
Total	50 µL

2. Add 50 µL of reaction mix to each well of the plate.
3. Add 50 µL 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.

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 µL
PBS	49 µL
Total	50 µL

2. Add 50 µL of reaction mix to each well of the plate.
3. Add 50 µL 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.