Signosis, Inc.
Innovative Plate Assay Solutions

AST Activity Assay Kit

Catalog # EA-7092 **(For Research Use Only)**

Introduction

Aspartate aminotransferase (AST) is an important enzyme that is involved in amino acid metabolism and energy production. This enzyme catalyzes the transfer of an amino group from aspartate to α-ketoglutarate, producing oxaloacetate and glutamate. AST is found in a variety of tissues throughout the body, including the liver, heart, skeletal muscle, and kidneys. Because AST is released into the bloodstream when cells are damaged, it is a clinically significant biomarker used to evaluate tissue injury. Elevated AST levels are commonly associated with liver diseases, such as hepatitis and cirrhosis, as well as heart and muscle damage.

Principle of the 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. AST activity is quantified by measuring the glutamate produced from the reaction using a paired enzyme reaction. Pyruvate is oxidized by glutamate oxidase to form glutamate byproducts and hydrogen peroxide. Then, the pyruvate level 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.

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

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

****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.

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.
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.