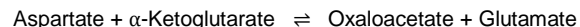


# Aspartate Aminotransferase (AST or SGOT) Activity Assay Kit

(Catalog #K753-100; 100 assays; Store kit at -20°C)

**I. Introduction:**

Aspartate aminotransferase (AST), also known as Glutamate-oxaloacetate transaminase (GOT) is a transaminase (EC 2.6.1.1) similar to the more liver specific alanine transaminase (ALT). Although commonly included clinically as part of a diagnostic liver function test, AST has a broader clinical utility since it may also be elevated in diseases affecting other organs, such as the heart or muscles in myocardial infarction, also in acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases and trauma. It catalyzes the reaction:



Diagnostically, it is almost always measured in units/liter (U/l). In BioVision's AST Assay Kit, an amino group is transferred of from aspartate to  $\alpha$ -ketoglutarate. The products of this reversible transamination reaction are oxaloacetate and glutamate. The glutamate is detected in a reaction that concomitantly converts a nearly colorless probe to color ( $\lambda_{\text{max}} = 450 \text{ nm}$ ). The kit provides a rapid, simple, sensitive and reliable test suitable as a high throughput activity assay of AST with a detection limit of 10 mU per well.

**II. Kit Contents:**

Components	100 assays	Cap Code	Part Number
AST Assay Buffer	25 ml	WM	K753-100-1
AST Enzyme Mix (lyophilized)	1 vial	Green	K753-100-2
AST Developer (lyophilized)	1 vial	Red	K753-100-3
AST Substrate (lyophilized)	1 vial	Orange	K753-100-4
Glutamate Standard (0.1M)	0.1 ml	Yellow	K753-100-5
AST Positive Control (lyophilized)	1 vial	Blue	K753-100-6

**III. Storage and Handling:**

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

**IV. Reagent Preparation:**

**AST Enzyme Mix:** Reconstitute with 220  $\mu\text{l}$  dH<sub>2</sub>O. Aliquot and store at -20°C. Use within two months.

**Developer:** Reconstitute with 820  $\mu\text{l}$  dH<sub>2</sub>O. Aliquot and store at -20°C. Use within two months.

**AST Substrate:** Reconstitute with 1.1 ml assay buffer. Store at -20°C. Use within two months.

**AST Positive Control:** Reconstitute with 100  $\mu\text{l}$  dH<sub>2</sub>O. Aliquot and store at -20°C. Use within two months. In the assay (optional), add 5  $\mu\text{l}$  positive control and adjust the volume to 50 $\mu\text{l}$ /well with Assay Buffer.

**V. AST Assay Protocol:**

**1. Standard Curve Preparation:**

Dilute 10  $\mu\text{l}$  of the 0.1M Glutamate Standard with 990  $\mu\text{l}$  Assay Buffer to generate 1 mM glutamate. Add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  into each well individually. Adjust the final volume to 50  $\mu\text{l}$ /well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glutamate Standard.

**2. Sample Preparations:**

Tissues (50 mg) or cells (1 x 10<sup>6</sup>) can be homogenized ~ 200  $\mu\text{l}$  of ice cold Assay Buffer then centrifuge (13,000 x g, 10 min) to remove insoluble material. Serum samples can be directly diluted in the Assay Buffer. Prepare test samples of up to 50  $\mu\text{l}$ /well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

- 3. Reaction Mix:** Mix enough reagent for the number of assays to be performed. For each well, prepare a total 100  $\mu\text{l}$  Reaction Mix.

AST Assay Buffer	80 $\mu\text{l}$
AST Enzyme Mix	2 $\mu\text{l}$
Developer	8 $\mu\text{l}$
AST Substrate	10 $\mu\text{l}$

Add 100  $\mu\text{l}$  of the Reaction Mix to each well containing the Samples, Standards, and Positive Controls (optional). Mix well.

- 1. Measurement:** Read OD 450 nm (A<sub>1</sub>) at T<sub>1</sub> (T<sub>1</sub> > 10 min) then again (A<sub>2</sub>) at T<sub>2</sub> after incubating the reaction at 37°C for 60 min (or longer if the AST activity is low), protect from light. The OD of the color generated by deamination of glutamate is  $\Delta A_{450 \text{ nm}} = A_2 - A_1$ . It is recommended that the user run the assay kinetically to choose A<sub>1</sub> and A<sub>2</sub> values which occur after the initial lag phase, during the linear range of color development. OD at A<sub>2</sub> should not exceed the highest OD generated in the standard curve.
- 4. Calculation:** Plot the glutamate standard curve and use the  $\Delta A_{450 \text{ nm}}$  to obtain B nmol of glutamate (amount of glutamate generated between T<sub>1</sub> and T<sub>2</sub> in the reaction wells). AST activity in the test samples can then be calculated:

$$\text{AST Activity} = \frac{B}{(T_2 - T_1) \times V} = \text{nmol/min/ml} = \text{mU/ml}$$

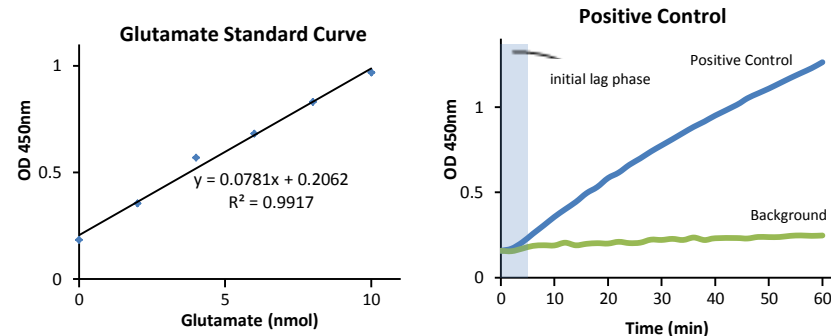
Where: **B** is the glutamate amount calculated from the Standard Curve (in nmol).

**T<sub>1</sub>** is the time of the first reading (A<sub>1</sub>) (in min).

**T<sub>2</sub>** is the time of the second reading (A<sub>2</sub>) (in min).

**V** is the original sample volume added into the reaction well (in ml).

One unit of AST is defined as the amount of AST which generates 1.0  $\mu\text{mol}$  of glutamate per minute at 37 °C.



**RELATED PRODUCTS:**

- Alanine Transaminase Assay Kit
- ADP/ATP Ratio Assay Kit
- Glucose Assay Kit
- Pyruvate Kinase Assay Kit
- Pyruvate Assay Kit
- Triglyceride Assay Kit
- Glycogen Assay Kit
- Glucose Assay Kit
- Fatty Acid Assay Kit
- Sarcosine assay Kit

- NADP/NADPH Quantitation Kit
- Glutamate Dehydrogenase Kit
- Fatty Acid Assay Kit
- LDH Quantification Kit
- para-Aminohippuric Acid Kit
- Lipase Assay Kit
- Lactate assay Kit
- Creatinine Assay Kit
- Cholesterol Assay Kit
- Uric Acid assay Kit

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

**GENERAL TROUBLESHOOTING GUIDE:**

<b>Problems</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed</li> <li>• Use fresh samples or store at correct temperatures until use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.