Aspartate Aminotransferase (AST or SGOT) Activity Colorimetric Assay Kit

(Catalog #BN00966; 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:

Aspartate + α-Ketoglutarate ⇌ Oxaloacetate + Glutamate

Diagnostically, it is almost always measured in units/liter (U/l). In Assay Genie's AST Assay Kit, an amino group is transferred of from aspartate to α-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 (λmax = 450 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	BN00966-1
AST Enzyme Mix (lyophilized)	1 vial	Green	BN00966-2
AST Developer (lyophilized)	1 vial	Red	BN00966-3
AST Substrate (lyophilized)	1 vial	Orange	BN00966-4
Glutamate Standard (0.1M)	0.1 ml	Yellow	BN00966-5
AST Positive Control (lyophilized)	1 vial	Blue	BN00966-6

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 µl dH_2O. Aliquot and store at -20°C. Use within two months.
Developer: Reconstitute with 820 µl dH_2O. 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 µl dH_2O. Aliquot and store at -20°C. Use within two months. In the assay (optional), add 5 µl positive control and adjust the volume to 50µl/well with Assay Buffer.

V. AST Assay Protocol:

1. **Standard Curve Preparation:**

   Dilute 10 µl of the 0.1M Glutamate Standard with 990 µl Assay Buffer to generate 1 mM glutamate. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust the final volume to 50 µl/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 10^6) can be homogenized ~ 200 µl 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 µl/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 µl Reaction Mix.

AST Assay Buffer	80 µl
AST Enzyme Mix	2 µl
Developer	8 µl
AST Substrate	10 µl

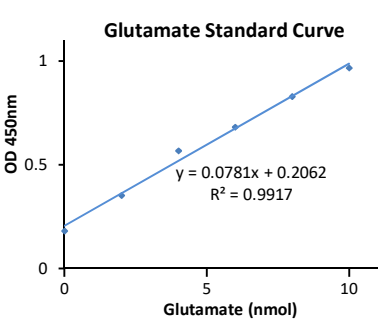
   Add 100 µl of the Reaction Mix to each well containing the Samples, Standards, and Positive Controls (optional). Mix well.

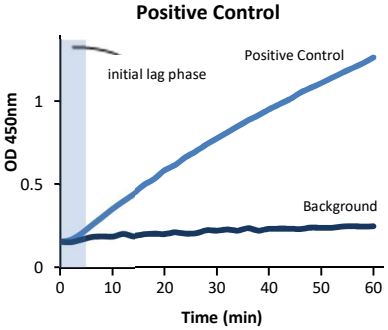
1. **Measurement:** Read OD 450 nm (A_1) at T_1 (T_1 > 10 min) then again (A_2) at T_2 after incubating the reaction at 37°C for 60 min (or longer if the AST activity is low), protect from light. The OD of the color generated by deamination of glutamate is ΔA450 nm = $A_2 - A_1$. It is recommended that the user run the assay kinetically to choose A_1 and A_2 values which occur after the initial lag phase, during the linear range of color development. OD at A_2 should not exceed the highest OD generated in the standard curve.
4. **Calculation:** Plot the glutamate standard curve and use the ΔA450 nm to obtain B nmol of glutamate (amount of glutamate generated between T_1 and T_2 in the reaction wells). AST activity in the test samples can then be calculated:

$$\text{AST Activity} = \frac{B}{(T2-T1)\times V} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** is the glutamate amount calculated from the Standard Curve (in nmol).
T_1 is the time of the first reading (A_1) (in min).
T_2 is the time of the second reading (A_2) (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 µmol of glutamate per minute at 37 °C.





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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.		

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