Product Information

Aspartate Aminotransferase (AST or SGOT) Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2171-100 100 assays	Cap Color	Part Number
AST Assay Buffer	25 ml	WM	K2171-C-1
AST Enzyme Mix (lyophilized)	1 vial	Green	K2171-C-2
AST Developer (lyophilized)	1 vial	Red	K2171-C-3
AST Substrate (lyophilized)	1 vial	Orange	K2171-C-4
Glutamate Standard (0.1M)	100 µl	Yellow	K2171-C-5
AST Positive Control (lyophilized)	1 vial	Blue	K2171-C-6

II. Introduction:

Aspartate aminotransferase (AST), also known as Glutamate-oxaloacetate transaminase (GOT) is a transaminase that is related to the more liver specific alanine transaminase (ALT). It is not only commonly used in liver function test but also for study in various diseases (e.g. myocardial infarction, acute pancreatitis and acute hemolytic anemia etc. It catalyzes the following reaction: Aspartate + α-Ketoglutarate ⇌ Oxaloacetate + Glutamate. In Aspartate Aminotransferase (AST or SGOT) Activity Colorimetric Assay Kit, an amino group is transferred from aspartate to α-ketoglutarate and produce oxaloacetate and glutamate which can be detected converts a nearly colorless probe to color (λmax = 450 nm). This kit offers a reliable and sensitive way for high throughput activity assay of AST with a detection limit of 10 mU per well.

III. Storage and Handling:

Store the kit at -20℃ 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℃. Use within two months.

Developer: Reconstitute with 820 µl dH_2O. Aliquot and store at -20℃. Use within two months.

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

AST Positive Control: Reconstitute with 100 µl dH_2O. Aliquot and store at -20℃. 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 0.1 M 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 (A1) at T1 (T1 > 10 min) then again (A2) at T2 after incubating the reaction at 37℃ 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 = A2 – A1. It is recommended that the user run the assay kinetically to choose A1 and A2 values which occur after the initial lag phase, during the linear range of color development. OD at A2 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 T1 and T2 in the reaction wells). AST activity in the test samples can then be calculated:

AST Activity = B/ [(T2−T1)× V] = nmol/min/ml = mU/ml

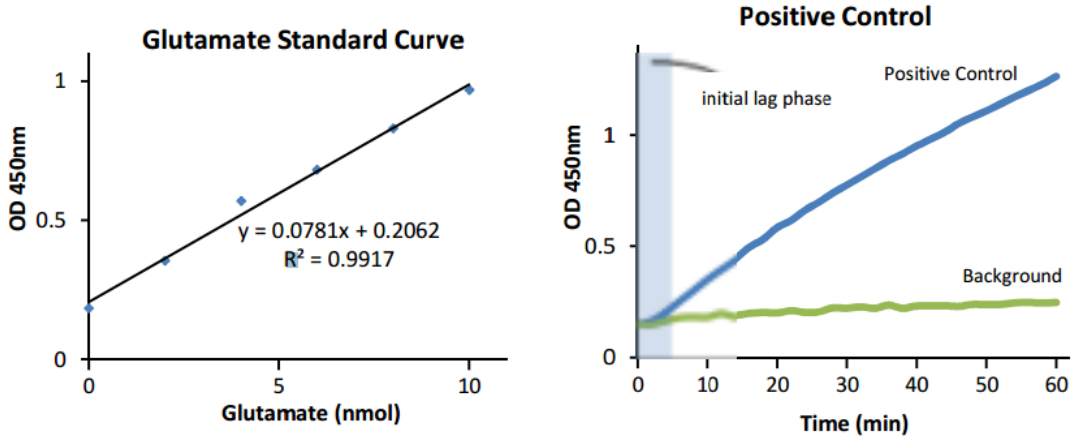
Where: B is the glutamate amount calculated from the Standard Curve (in nmol).

T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (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℃.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples

erratic readings	• Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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