

Product Information

Aspartate Aminotransferase (AST) Activity Assay Kit

Catalog Number **MAK055**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

Aspartate Aminotransferase (AST), formerly called serum glutamate-oxaloacetate transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes the conversion of aspartate and α -keto-glutarate to oxaloacetate and glutamate. Similar to Alanine Aminotransferase (ALT), AST levels in blood are commonly used as a marker for liver function. However, AST has a broader tissue distribution than ALT and perturbations in AST levels can occur in response to diseases or injuries in multiple tissues including skeletal and heart.

The Aspartate Aminotransferase Activity Assay kit provides a simple and direct procedure for measuring AST activity in a variety of samples. In this kit, the transfer of an amino group from aspartate to α -keto-glutarate results in the generation of glutamate, resulting in the production of a colorimetric (450 nm) product proportional to the AST enzymatic activity present. One unit of AST is the amount of enzyme that will generate $1.0\text{ }\mu\text{mole}$ of glutamate per minute at pH 8.0 at $37\text{ }^{\circ}\text{C}$.

Components

The kit is sufficient for 100 assays in 96 well plates.

AST Assay Buffer, Catalog Number MAK055A	25 mL
AST Enzyme Mix Catalog Number MAK055B	1 vL
AST Developer Catalog Number MAK055C	1 vL
AST Substrate Catalog Number MAK055D	1 vL
Glutamate Standard, 0.1 M Catalog Number MAK055E	0.1 mL
AST Positive Control Catalog Number MAK055F	1 vL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

AST Assay Buffer – Allow buffer to come to room temperature before use.

AST Enzyme Mix – Reconstitute with $220\text{ }\mu\text{L}$ of water. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution.

AST Developer – Reconstitute with $820\text{ }\mu\text{L}$ of water. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution.

AST Substrate – Reconstitute with 1.1 mL of AST Assay Buffer. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution.

AST Positive Control – Reconstitute with $100\text{ }\mu\text{L}$ of water. Mix well by pipetting, then aliquot and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice and storage at $-20\text{ }^{\circ}\text{C}$, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

Glutamate Standards for Colorimetric Detection

Dilute 10 μL of the 0.1 M Glutamate Standard solution with 990 μL of the AST Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add AST Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

Tissue (50 mg) or cells (1×10^6) can be homogenized in 200 μL of ice-cold AST Assay Buffer. Centrifuge the samples at $13,000 \times g$ for 10 minutes to remove insoluble material.

Serum samples can be directly added to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 μL with AST Assay Buffer.

For the positive control (optional), add 5 μL of the AST positive control solution to wells and adjust to 50 μL with the AST Assay Buffer.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 100 μL of the Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Master Reaction Mix
AST Assay Buffer	80 μL
AST Enzyme Mix	2 μL
AST Developer	8 μL
AST Substrate	10 μL

2. Add 100 μL of the Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation
3. Incubate the plate at 37 $^{\circ}\text{C}$. After 2–3 minutes, take the initial measurement (T_{initial}). Measure the absorbance at 450 nm at the initial time ($(A_{450})_{\text{initial}}$).
Note: It is essential ($(A_{450})_{\text{initial}}$ is in the linear range of the standard curve.
4. Continue to incubate the plate at 37 $^{\circ}\text{C}$ taking measurements (A_{450}) every 5 minutes. Protect the plate from light during the incubation.
5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (10 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
6. The final measurement [$(A_{450})_{\text{final}}$] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final} .
Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement $[(A_{450})_{\text{final}}]$ obtained for the 0 (blank) Glutamate standard from the final measurement $[(A_{450})_{\text{final}}]$ of the standards. Background values can be significant. Plot the Glutamate standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from T_{initial} to T_{final} for the samples.

$$\Delta A_{450} = (A_{450})_{\text{final}} - (A_{450})_{\text{initial}}$$

Compare the ΔA_{450} of each sample to the standard curve to determine the amount of glutamate generated by the aminotransferase assay between T_{initial} and T_{final} (B).

The AST activity of a sample may be determined by the following equation:

$$\text{AST Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of glutamate generated between T_{initial} and T_{final} .

Reaction Time = $T_{\text{final}} - T_{\text{initial}}$ (minutes)

V = sample volume (mL) added to well

AST activity is reported as nmole/min/mL = milliunit/mL
One unit of AST is the amount of enzyme that will generate 1.0 μmole of glutamate per minute at pH 8.0 at 37 °C.

Example:

Glutamate amount (B) = 5.84 nmole

First reading (T_{initial}) = 3 minute

Second reading (T_{final}) = 32 minutes

Sample volume (V) = 0.01 mL

Sample dilution is 1

AST activity is:

$$\frac{5.84 \times 1}{(32-3) \times 0.01} = 20.14 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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