ab138878

Aspartate Aminotransferase Activity Assay Kit (Fluorometric)

Instructions for Use

For measurement of Aminotransferase activity (AST) in various biological samples.

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Aspartate aminotransferase (AST), also called serum glutamic oxaloacetic transaminase (GOT), is a member of transferase family. It catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and is an important enzyme in amino acid metabolism. AST is found in many body tissues such as liver, heart, muscle, kidneys, brain. In healthy subjects, serum AST levels are low. However, when cells are damaged, such as acute and chronic hepatitis, obstructive jaundice, carcinoma of liver, myocardial infarction, AST may leak into the blood stream and the AST levels are significantly elevated. Therefore, determination of serum AST level has great clinical and diagnostic significance.

ab138878 provides a quick and sensitive method for the measurement of AST in various biological samples. Aspartate transaminase catalyzes the reaction of aspartate and α-ketoglutarate to oxaloacetate and glutamate:

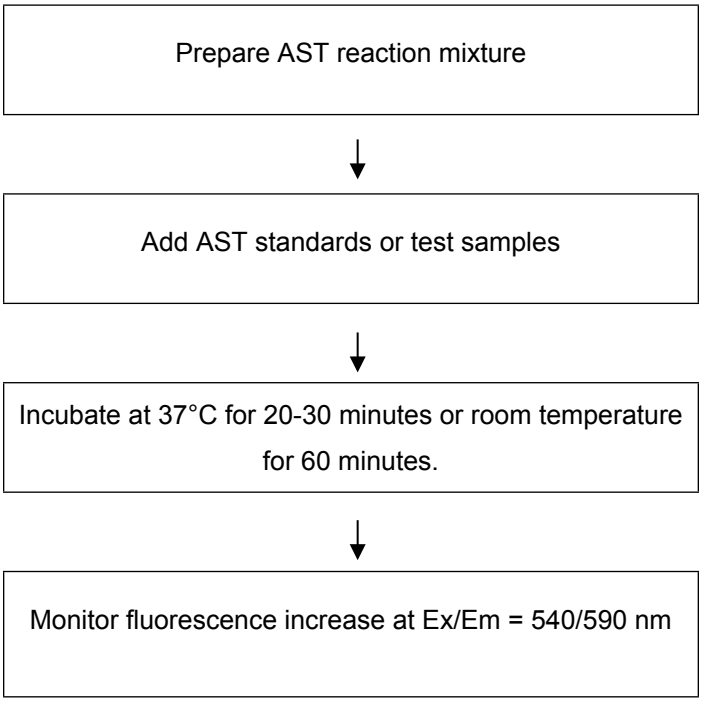
Aspartate (Asp) + α-ketoglutarate ⇌ oxaloacetate + glutamate (Glu)

The product L-glutamate is measured by the generation of a red fluorescent product through an enzyme coupled reaction cycle. The signal can be read by a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm). ab138878 can detect as little as 2 mU/ml AST in a 100 µl reaction

volume. The assay is robust and can be readily adapted for a wide variety of applications.

2. Protocol Summary

Summary for One 96 well Plate



Note: Thaw one bottle each of AST Assay Enzyme Mixture and AST Assay Buffer at room temperature before starting the experiment.

3. Kit Contents

Components	Amount
AST Assay Enzyme Mixture	1 vial (lyophilized)
AST Assay Buffer	1 bottle (10 ml)
AST Positive Control	1 vial (10 U)
NAD	1 vial

4. Storage and Handling

Keep at NAD, AST Assay Enzyme Mixture and AST Assay Buffer -20°C. AST Positive Control should be stored at -20°C (store in the dark and avoid freeze / thaw cycle). The Reaction mixture (prepared by mixing AST Assay Enzyme Mixture and AST Assay Buffer, see Preparation of AST Reaction Mixture) is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light.

5. Additional Materials Required

- 96 or 384 well microplates: Solid tissue culture microplates with black walls and clear bottom.
- Fluorescence microplate reader
- DPBS (Dulbecco's Phosphate Buffered Saline)
- 0.1% BSA

6. Assay Protocol

Note: This protocol is for one 96 well plate.

A. Preparation of AST Reaction Mixture

1. Add 100 µL of ddH_2O into the vial of NAD to have 100X NAD solution.

2. Add 10 ml of AST Assay Buffer into the bottle of AST Assay Enzyme Mixture and mix well.
3. Add whole vial of 100X NAD solution (from Step 1) into the AST Enzyme Mixture solution (from Step 2) to have AST assay mixture.

   Note: This AST reaction mixture is enough for two 96 well plate. It is unstable at room temperature and should be used promptly within 2 hours and avoid exposure to light.

   Note2: Alternatively, one can make a 50X of AST Enzyme Mixture stock solution by adding 200 µL of H_2O into the AST Assay Enzyme Mixture, and then prepare the AST assay mixture by mix the stock solution with assay buffer and 100x NAD solution proportionally. Aliquot and store the unused 50X AST Enzyme Mixture

stock solution and 100X NAD solution at -20°C and avoid freeze-thaw cycles.

B. Preparation of serial dilutions (AST standard (1 to 300 mU/ml)

1. Add desired amount of AST Positive Control to DPBS Buffer to make 100 U/ml AST standard solution.

   Note: The unused AST Positive Control should be always stored at -20 °C (store in the dark and avoid freeze / thaw cycle).

2. Add 3 µl of 100 U/ml AST standard solution into 997 µl DPBS buffer with 0.1% BSA to generate 300 mU/ml AST standard solution.

3. Take 300 µl of 300 mU/ml AST standard solution to perform 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3 and 0 mU/ml serial dilutions of AST standard. The dilutions should be made in DPBS buffer with 0.1 % BSA.

4. Add serial dilutions of AST standard and AST containing test samples into a solid black 96-well microplate as described in Tables 1 and 2.

Note: Dilute the test samples to the concentration range in DPBS with 0.1% BSA if needed.

Table 1 Layout of AST standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	...							
AST1	AST1	...	...								
AST2	AST2										
AST3	AST3										
AST4	AST4										
AST5	AST5										
AST6	AST6										
AST7	AST7										

Note: AST=Standards, BL=Blank Control, TS=Test Samples

Table 2 Reagent composition for each well

AST Standards	**Blank Control**	**Test sample**
Serial Dilutions*: 50 µl	DPBS with 0.1% BSA: 50 µl	50 µl

Note: Add the serially diluted standards from 0.3 mU/ml to 300 mU/ml into wells from AST1 to AST 7 in duplicate.

C. Run AST Assay:

1. Add 50 µl of AST Reaction Mixture to each well of standard, blank control or test sample to make the total assay volume of 100 µl/well.

   Note: For a 384-well plate, add 25 µl of sample and 25 µl of AST Reaction Mixture into each well.

2. Incubate at 37 °C for 20-30 minutes or room temperature for 60 minutes, protected from light.

   Note: The background of Blank Control increases with time and temperature. The background will be high if incubate at 37 °C over 30 minutes.

3. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 530 - 570/590 - 600 nm (optimal Ex/Em = 540/590 nm, cut off at 570 nm).

7. Data Analysis

The fluorescence in blank wells (with the DPBS buffer with 0.1%BSA only) is used as a control and is subtracted from the values for those wells with the AST reactions.

Note: The fluorescence background increases with time, thus it is important to subtract the intensity value of the blank wells from that of each data point.

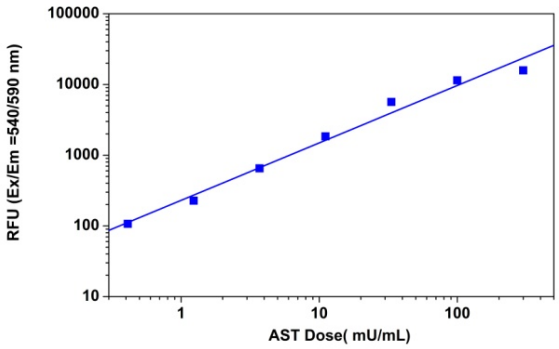


Figure 1. AST dose response was measured with ab138878 in a 96-well black plate using a fluorescence microplate reader. As low as 2 mU/ml AST can be detected with 60 min incubation (n=3) at room temperature.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp