Ab105135 Aspartate Aminotransferase Activity Assay Kit

For the rapid, sensitive and accurate measurement of Aspartate aminotransferase activity in various samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Components and Storage

Items	Quality
Assay Buffer XIX/Assay Buffer	25 mL
AST Enzyme Mix/Enzyme Mix (Lyophilized)	1 vial
Developer Solution III/Developer (Lyophilized)	1 vial
AST Substrate/Substrate (Lyophilized)	1 vial
Glutamate Standard/Glutamate Standard (0.1M)	0.1 mL
AST Positive Control/Positive Control (Lyophilized)	1 vial

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

AST Enzyme Mix/Enzyme mix: Reconstitute with 220 µl dH_2O. Aliquot and store at -20°C. Use within two months.

Developer Solution III/Developer: Reconstitute with 820 µl dH_2O. Aliquot and store at -20°C. Use within two months

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

AST Positive Control/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 50 µl/well with Assay Buffer XIX/Assay Buffer.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

Assay Procedure

1. Sample Preparation:

Tissues (50 mg) or cells (1 x 10^6) can be homogenized ~200 µl of ice cold Assay Buffer XIX/Assay Buffer then centrifuge (13,000 x g, 10 min) to remove insoluble material.

Serum samples can be directly diluted in the Assay Buffer XIX/Assay Buffer.

- Prepare test samples of up to 50 µl/well with Assay Buffer XIX/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*.

Recommended input per well

Biological fluids: 5-20 uL

Number of lysed cells: 0.5-2x10e5

Cell culture supernatants: 5-20 uL

Tissue lysate (protein mass): >5 ug

2. Standard Curve Preparation:

Dilute 10 µl of the Glutamate Standard/0.1M Glutamate Standard with 990 µl Assay Buffer XIX/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 XIX/Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glutamate Standard.

Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix.

Assay Buffer XIX/Assay Buffer 80 µl
AST Enzyme Mix/Enzyme Mix 2 µl
Developer Solution III/Developer 8 µl
AST Substrate/Substrate 10 µl

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

4. Read OD_{450nm} (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:

$$\Delta A_{450nm} = A_2 - A_1.$$

Note: 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. Consider T2 (60 mins) values of standards for calculation. The values of the standard should be stable after 10 mins.

Data Analysis

Average the duplicate reading for each standard and sample.

Subtract the mean absorbance value of the blank from all standard and sample readings. This is the corrected absorbance.

Plot the glutamate standard curve and use the ΔA_{450nm} to obtain B nmol of glutmate.

Aspartate aminotransferase (AST) activity in the test samples can then be calculated:

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

Where:

B is the glutamate amount (nmol) calculated from the Standard Curve

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).

D is the sample dilution factor.

One unit of AST is defined as the amount of AST which generates 1.0 µmol of glutamate per minute at 37 °C.

For example, if you added 10 µl of undiluted cell lysate and make up the volume in the 96 well up to 50 µl using assay buffer, your V is 0.01 and dilution factor is 1. Alternatively, if you added 10 µl of (1:10 diluted cell lysate) and make up the volume in the 96 well up to 50 µl using assay buffer, your V is 0.01 and dilution factor is 10.

Troubleshooting

Problem	Reason	Solution
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
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
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)***
	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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)