ab105135

Aspartate Aminotransferase Activity Assay Kit

Instructions for Use

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.

Version 3 Last Updated: 9 October 2014
# Table of Contents

1. Overview 3
2. Protocol Summary 4
3. Components and Storage 5
4. Assay Protocol 7
5. Data Analysis 9
6. Troubleshooting 11
1. Overview

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, Aspartate aminotransferase 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:

\[
\text{Aspartate} + \alpha - \text{Ketoglutarate} = \text{Oxaloacetate} + \text{Glutamate}
\]

Diagnostically, it is almost always measured in units/liter (U/L). In Abcam’s Aspartate Aminotransferase Activity Assay Kit an amino group is transferred from aspartate to \(\alpha\)-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 \((\lambda_{\text{max}} = 450 \text{ nm})\).

The kit provides a rapid, simple, sensitive and reliable test suitable as a high throughput activity assay of Aspartate aminotransferase with a detection limit of 10 mU per well.
2. Protocol Summary

Sample Preparation

Standard Curve Preparation

Prepare and Add Reaction Mix

Measure Optical Density
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>25 mL</td>
</tr>
<tr>
<td>Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Developer (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Substrate (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Glutamate Standard (0.1M)</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Positive Control (Lyophilized)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

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.

ENZYME MIX: Reconstitute with 220 µl dH₂O. Aliquot and store at -20°C. Use within two months.

DEVELOPER: Reconstitute with 820 µl dH₂O. Aliquot and store at -20°C. Use within two months

SUBSTRATE: Reconstitute with 1.1 ml assay buffer. Store at -20°C. Use within two months.
POSITIVE CONTROL: Reconstitute with 100 μl dH₂O. 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.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker
4. Assay Protocol

1. Sample Preparation:
   a. **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.

   b. **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.*

   **Recommended input per well**
   - Biological fluids: 5-20 μL
   - Number of lysed cells: 0.5-2x10^5
   - Cell culture supernatants: 5-20 μL
   - Tissue lysate (protein mass): >5 μg

2. 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.
3. Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 100 \( \mu l \) Reaction Mix.

- Assay Buffer 80 \( \mu l \)
- Enzyme Mix 2 \( \mu l \)
- Developer 8 \( \mu l \)
- Substrate 10 \( \mu l \)

Add 100 \( \mu l \) 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\(^\circ\)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.
5. Data Analysis

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

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

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

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).

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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Assay buffer at wrong temperature</td>
<td>Assay buffer must not be chilled - needs to be at RT</td>
</tr>
<tr>
<td>Protocol step missed</td>
<td>Protocol step missed</td>
<td>Re-read and follow the protocol exactly</td>
</tr>
<tr>
<td>Plate read at incorrect</td>
<td>Plate read at incorrect wavelength</td>
<td>Ensure you are using appropriate reader and filter settings (refer to datasheet)</td>
</tr>
<tr>
<td>wavelength</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsuitable microtiter plate</td>
<td>Unsuiltable microtiter plate for assay</td>
<td>Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells</td>
</tr>
<tr>
<td>Unexpected results</td>
<td>Measured at wrong wavelength</td>
<td>Use appropriate reader and filter settings described in datasheet</td>
</tr>
<tr>
<td>Samples contain impeding</td>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td>type</td>
<td>Unsuitable sample type</td>
<td>Use recommended samples types as listed on the datasheet</td>
</tr>
<tr>
<td>Sample readings are outside</td>
<td>Sample readings are outside linear</td>
<td>Concentrate/ dilute samples to be in linear range</td>
</tr>
<tr>
<td>linear range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples with inconsistent readings</td>
<td>Unsuitable sample type</td>
<td>Refer to datasheet for details about incompatible samples</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Samples prepared in the wrong buffer</td>
<td>Use the assay buffer provided (or refer to datasheet for instructions)</td>
<td></td>
</tr>
<tr>
<td>Samples not deproteinized (if indicated on datasheet)</td>
<td>Use the 10kDa spin column <em>(ab93349)</em></td>
<td></td>
</tr>
<tr>
<td>Cell/ tissue samples not sufficiently homogenized</td>
<td>Increase sonication time/ number of strokes with the Dounce homogenizer</td>
<td></td>
</tr>
<tr>
<td>Too many freeze-thaw cycles</td>
<td>Aliquot samples to reduce the number of freeze-thaw cycles</td>
<td></td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
<td></td>
</tr>
<tr>
<td>Samples are too old or incorrectly stored</td>
<td>Use freshly made samples and store at recommended temperature until use</td>
<td></td>
</tr>
<tr>
<td>Lower/ Higher readings in samples and standards</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td>Out-of-date kit or incorrectly stored reagents</td>
<td>Always check expiry date and store kit components as recommended on the datasheet</td>
<td></td>
</tr>
<tr>
<td>Reagents sitting for extended periods on ice</td>
<td>Try to prepare a fresh reaction mix prior to each use</td>
<td></td>
</tr>
<tr>
<td>Incorrect incubation time/ temperature</td>
<td>Refer to datasheet for recommended incubation time and/ or temperature</td>
<td></td>
</tr>
<tr>
<td>Incorrect amounts used</td>
<td>Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard curve is not linear</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
</tr>
<tr>
<td></td>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
</tr>
<tr>
<td></td>
<td>Air bubbles in wells</td>
<td>Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates</td>
</tr>
<tr>
<td></td>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
</tr>
<tr>
<td></td>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
</tr>
<tr>
<td></td>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
</tr>
</tbody>
</table>

13
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