ab105135 – Aspartate Aminotransferase Activity Assay Kit

For rapid, sensitive and accurate measurement of AST activity in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab105135 (use http://www.abcam.cn/ab105135 for China, or http://www.abcam.co.jp/ab105135 for Japan)

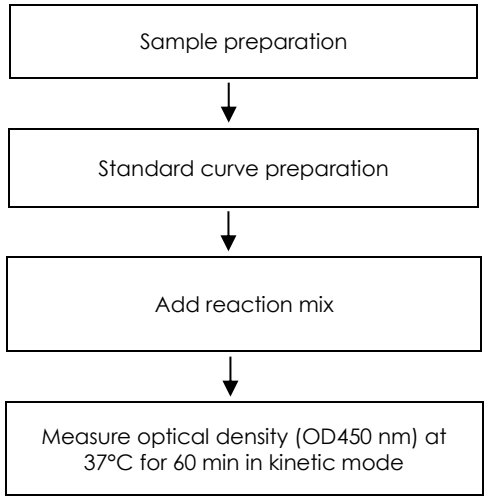
Background:

Aspartate Aminotransferase Activity Assay Kit (ab105135) provides a simple, rapid and reliable, automation-ready procedure for measuring aspartate aminotransferase (AST or SGOT) activity in tissue extracts, cell lysate, plasma, serum, and other biologicals.

Aspartate aminotransferase catalyses the reaction converting aspartate and a-ketoglutarate to oxaloacetate and glutamate. The glutamate is detected in a reaction that concomitantly converts a nearly colorless probe to a brightly colored chromophore detectable by absorbance (λ_{max} = 450 nm).

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- set plate reader/incubator to 37°C
- Solubilize AST Enzyme Mix, AST Substrate and AST Positive Control. Thaw Developer Solution III, Glutamate Standard and Assay Buffer 19(aliquot if necessary);
- Prepare samples in duplicate
- Prepare standard curve
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare Reaction Mix (Number samples + standards + 1).
- Add 100 µL Reaction Mix to each well.
- Measure plate at OD 450 nm in kinetic mode at 37°C for 60 mins.

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 19	25 mL	-20°C	-20°C
AST Enzyme Mix	1 vial	-20°C	-20°C
Developer Solution III	1 vial	-20°C	-20°C
AST Substrate	1 vial	-20°C	-20°C
Glutamate Standard	0.1 mL	-20°C	-20°C
AST Positive Control	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 19 was previously labeled as Assay Buffer XIX and Assay Buffer. The composition has not changed.

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance (OD) at 450 nm
- 96 well clear plate with clear flat bottom
- Orbital shaker
- Microcentrifuge
- Dounce homogenizer (if using cells or tissue)
- 1 x PBS pH 7.4
- MilliQ water or other type of double distilled/deionized water (ddH2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

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

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

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

AST 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 AST positive control and adjust the volume to 50 µl/well with Assay Buffer 19.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

1. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples at -80°C. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

Cells (adherent or suspension) samples:
1. Harvest the number of cells necessary for each assay
2. Wash cells in cold PBS.
3. Resuspend cells in 200 µL of Assay Buffer 19.
4. Homogenize cells with a Dounce homogenizer sitting on ice.
5. Centrifuge sample for 10 minutes at 4°C at 13,000 x g using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Tissue Samples:
1. Harvest the amount of tissue necessary for each assay (initial recommendation = 50 mg (equivalent to >5 µg/well).
2. Wash tissue in cold PBS.
3. Resuspend tissue in 200 µL of Assay Buffer 19.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 - 15 passes.
5. Centrifuge samples for 10 minutes at 4°C at 13,000 x g using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Liquid Samples (serum and other biological fluids): Samples can be diluted in Assay Buffer 19 for testing. Biological samples such as serum should be diluted 1:10 and volumes in the range of 5 – 50 µL used.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare 1mM dilution of Glutamate Standard as follows:

1. Add 10 µL of undiluted Glutamate Standard to 990 µL Assay Buffer 19. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.
2. Add 0, 2, 4, 6, 8, 10 µl into each well individually and adjust the final volume to 50 µl/well with Assay Buffer 19 to generate 0, 2, 4, 6, 8, 10 nmol/well of Glutamate Standard, or prepare the standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for duplicate standard curves).

Standard #	Volume of 1 mM Standard (µL)	Assay Buffer 19 (µL)	Final volume standard in well (µL)	End Glutamate Amount (nmoles/well)
1	0	125	50	0
2	5	120	50	2
3	10	115	50	4
4	15	110	50	6
5	20	105	50	8
6	25	100	50	10

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

1. Set up Reaction wells:
   Standard wells = 50 µL standard dilutions.
   Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 19).
   Background wells = 50 µL Assay Buffer 19.
2. Each well (standards, samples, and controls) requires 100 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:
   X µL component x (Number reactions +1).

Component	Colorimetric Assay Reaction Mix (µL)
Assay Buffer 19	80
AST Enzyme Mix	2
Developer Solution III	8
AST Substrate	10

3. Mix Master Reaction Mix by inversion. Add 100 µL of the Master Reaction Mix to each well and mix. Use a clean tip for each well.
4. Immediately start reading plate in kinetic mode, taking readings at OD_{450nm} every 1-2 minutes at 37°C for 60 min (or longer if the AST activity is low).*

* 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. If kinetic readings are not possible, read OD450 nm (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 OD at A_2 should not exceed the highest OD generated in the standard curve. Consider T_2 (60 mins) values of standards for calculation. The values of the standard should be stable after 10 mins.

Calculations:

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank from all standard readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of glutamate(in nmoles).
4. Calculate the equation of the standard curve using a linear regression and determine the slope.
5. Determine the ΔAbsorbance for each sample by finding two timepoints in the linear range (T_1 and T_2) and determining $\Delta A_{450\,nm} = A_2 - A_1$ for the respective time points.
6. Apply the $\Delta A_{450\,nm}$ to the standard curve to find (B) amount of glutamate (in nmoles) produced by AST.

$$B = \left(\frac{\Delta A_{\,450\,nm} - (y - intercept)}{Slope}\right)$$

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

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

Where:

B = glutamate amount (in nmoles) calculated from the Standard Curve

T_1 = the time of the first reading (A_1) (in minutes).

T_2 = the time of the second reading (A_2) (in minutes).

V = the original sample volume added into the reaction well (in ml).

D = the sample dilution factor (before addition to the well).

One unit of AST is defined as the amount of AST which generates 1.0 µmole 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.

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
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