Aspartate (Asp) Assay Kit

Catalog No.: abx294432

Size: 96 tests

Detection Range: 1.7 µmol/L – 471.7 µmol/L

Sensitivity: 1.7 µmol/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of Aspartate concentration in serum, plasma, tissue homogenates, and cell lysates.

Introduction

Abbexa's Aspartate (Asp) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Aspartate concentration. Enzymes provided in the kit convert Aspartate to glutamic acid, which in turn is broken down to produce an orange product with an absorbance maximum at 450 nm. The intensity of the color is proportional to the Aspartate concentration, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 55 ml
3. Substrate: 0.5 ml
4. Catalyst: 2 × 0.48 ml
5. Enzyme Reagent: 2 × 0.48 ml
6. Oxidizing Reagent: 2 vials
7. Chromogenic Reagent: 5 ml
8. Accelerating Reagent: 0.5 ml
9. Standard (500 µmol/L): 5 ml
10. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Ultrafiltration tube (3 kDa)
3. Double-distilled water
4. PBS (0.01M, pH 7.4)
5. Pipette and pipette tips
6. Centrifuge and centrifuge tubes
7. Mechanical homogenizer
8. Sonicator
9. Vortex mixer
10. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Take the prepared serum/plasma and filter through a 3 kDa ultrafiltration tube. Centrifuge the filtrate at 12,000 × g for 10 minutes, then take the supernatant for detection.
- **Tissue homogenates:** Carefully weigh at least 20 mg of tissue. Wash the tissue in cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add into 180 µl of Buffer Solution. Homogenize manually, using a mechanical homogenizer, at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant and filter through a 3 kDa ultrafiltration tube. Centrifuge the filtrate at 12,000 × g for 10 minutes. Take the supernatant for detection and assay immediately.
- **Cell lysates:** Collect at least 1×10^6 cells for analysis. Wash in cold PBS (0.01 M, pH 7.4). Per 1×10^6 cells, add into 300 µl of Buffer Solution. Homogenize using a sonicator at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant and filter through a 3 kDa ultrafiltration tube. Centrifuge the filtrate at 12,000 × g for 10 minutes. Take the supernatant for detection and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Mouse liver tissue homogenate	3 – 4
10% Mouse heart tissue homogenate	3 – 4
10% Rat liver tissue homogenate	3 – 4
10% Rat kidney tissue homogenate	3 – 4
10% Rat heart tissue homogenate	3 – 4
Dog serum	1
Horse plasma	1
1×10^6 CHO cells	1
1×10^6 Jurkat cells	1
1×10^6 239T cells	1
1×10^6 Molt-4 cells	1
1×10^6 HL-60 cells	1
1×10^6 THP-1 cells	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Oxidizing Working Solution:** Reconstitute 1 vial of Oxidizing Reagent with 2.5 ml double-distilled water. Mix thoroughly until fully dissolved. Unused Oxidizing Working Solution can be stored at -20°C in the dark for up to 3 days.
- **Assay Working Solution:** Prepare enough Assay Working Solution as required for the wells tested. Mix Buffer Solution, Substrate, Catalyst, Enzyme Reagent, Oxidizing Working Solution and Accelerating Reagent at a ratio of 353:5:20:20:100:2. For example, prepare 500 µl Assay Working Solution by mixing thoroughly 353 µl Buffer Solution, 5 µl Substrate, 20 µl Catalyst, 20 µl Enzyme Reagent, 100 µl Oxidizing Working Solution, and 2 µl Accelerating Reagent. Prepare just before use and keep the prepared solution on ice, protected from light.
- **Control Solution:** Prepare only as much Control Solution as required for the number of control wells tested. To prepare 500 µl Control Solution, mix thoroughly 378 µl Buffer Solution, 20 µl Enzyme Reagent, 100 µl Oxidizing Working Solution, and 2 µl Accelerating Reagent. Prepare just before use and keep the prepared solution on ice in the dark.
- **Standards:** Label 7 tubes with 500 µmol/L, 400 µmol/L, 350 µmol/L, 300 µmol/L, 200 µmol/L, 150 µmol/L, and 100 µmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (µmol/L)	500	400	350	300	200	150	100
Standard (500 µmol/L) (µl)	200	160	140	120	80	60	40
Buffer Solution (µl)	0	40	60	80	120	140	160

For the blank, use pure Buffer Solution. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark positions on the 96-well microplate for each standard, sample, and control. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 20 µl of sample to each sample well and corresponding control well.
3. Add 20 µl of each standard dilution to the corresponding standard wells.
4. Add 200 µl of Assay Working Solution to the standard and sample wells.
5. Add 200 µl of Control Solution to the control wells.
6. Add 40 µl of Chromogenic Reagent to all wells.
7. Mix the well contents by tapping the plate, or shaking with a microplate shaker, for at least 5 seconds. Cover the plate with a plate sealer and incubate at 37°C for 40 minutes in the dark.
8. Measure the OD of each well with a microplate reader at 450 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Aspartate in each sample well can be derived with the following formulae:

1. Serum and Plasma samples:

$$\text{Aspartate (µmol/L)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b})}{\text{a}} \times \text{F}$$

2. Tissue samples:

$$\text{Aspartate (µmol/kg tissue)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Homogenate}}}{\text{a} \times \text{W}_{\text{Tissue}}} \times \text{F}$$

3. **Tissue samples:**

$$\text{Aspartate (µmol/10}^6\text{ cells)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}} - \text{b}) \times \text{V}_{\text{Homogenate}}}{\text{a} \times \text{N}_{\text{Cells}}} \times \text{F}$$

where:

$\text{OD}_{\text{Sample}}$	OD value of sample
$\text{OD}_{\text{Control}}$	OD value of control
$\text{V}_{\text{Homogenate}}$	Volume of tissue homogenate tested (ml)
W_{Tissue}	Wet weight of tissue used in tissue homogenate preparation (g)
N_{Cells}	Number of cells used in cell lysate preparation ($N \times 10^6$)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.