Technical Bulletin

Aspartate Assay Kit

Catalogue number MAK495

Product Description

Aspartate, a nonessential amino acid, is a precursor to several other amino acids and is an excitatory neurotransmitter. Aspartate is involved in the urea cycle and gluconeogenesis.

The Aspartate Assay Kit provides a simple, direct, and automation-ready procedure for measuring aspartate concentration. Aspartate is converted into pyruvate which is then oxidized with the conversion of the dye into a colored and fluorescent form. The color intensity of the oxidized dye at 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/λ_{Em} = 585 nm is directly proportional to the aspartate concentration in the sample.

The linear detection range of the kit is 2 to 400 µM aspartate for colorimetric assays and 1 to 50 µM for fluorometric assays. The kit is suitable for Aspartate activity determination in plasma, serum, tissue and culture media, as well as for studying the effects of drugs on aspartate metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Developer 10 mL
  Catalogue Number MAK495A
- AST Enzyme 240 µL
  Catalogue Number MAK495B
- Dye reagent 120 µL
  Catalogue Number MAK495C
- Cosubstrate 600 µL
  Catalogue Number MAK495D
- ODC Enzyme 120 µL
  Catalogue Number MAK495E
- Aspartate Standard (10 mM)
  Catalogue Number MAK495F 400 µL

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example: multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- Dounce tissue grinder set.
  (Catalogue Number D9063 or equivalent)
- Phosphate Buffered Saline (PBS)
  (Catalogue Number P3813 or equivalent)
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Note: The assay requires 30 min when performed at 37 °C or 60 min if performed at Room Temperature (25 °C).
Developer: Equilibrate Developer to desired assay temperature.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

For tissue and cell Samples:

1. Homogenize the tissue or cell Samples (2 x10^6) in 100 µL PBS.
2. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay.

For serum and plasma Samples:

1. Samples should be deproteinated using a 10 kDa spin filter (for example: Amicon Ultra-0.5).

Note: An internal Standard should be used for serum and plasma Samples, and it is highly recommended that the fluorescent assay be used due to low aspartate concentrations.

Note: If planning to measure aspartate in culture media, avoid media with high pyruvate concentrations if possible (for example: DMEM, L-15, F12, etc.).

2. Transfer 25 µL of each Sample to two separate wells in a 96 well plate (each Sample requires a Sample Blank).

Note: Samples requiring an internal Standard will need three separate reactions:

1. Sample Plus Standard

2. Sample alone

3. Sample Blank

Colorimetric Standard Curve Preparation

Note: If assaying culture media with phenol red, dilute the Aspartate Standard in culture media.

1. Prepare a 400 µM Standard by mixing 10 µL of the 10 mM Standard with 240 µL of purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1. Preparation of Aspartate Colorimetric Standards

No.	400 µM Standard	Purified water	Aspartate (µM)
1	100 µL	0 µL	400
2	60 µL	40 µL	240
3	30 µL	70 µL	120
4	0 µL	100 µL	0

3. Mix well and transfer 25 µL of each standard into separate wells of a clear flat-bottom 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare a 40 µM Standard by diluting the Standards prepared in Colorimetric Standard Curve Preparation (Table 1.) 1:10 in purified water.
2. Mix well and transfer 25 µL of each standard into separate wells of a black 96-well plate.

Preparation of internal standards:

1. For the internal standard, prepare a 100 µM aspartate standard by mixing 5 µL of the 10 mM Standard with 495 µL purified water.
2. For the Sample plus Standard well, add 5 µL 100 µM aspartate and 25 µL sample.
3. For the Sample and Sample Blank wells, add 5 µL purified water and 25 µL sample.

Working Reagent Preparation

Mix enough reagents for the number of assays to be performed. For each Sample Plus Standard and Sample well, prepare 94 µL of Working Reagent according to Table 2. For each Sample Blank well, prepare 92 µL of Blank Control Reagent according to Table 2.

Table 2.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Control Reagent
Developer	85 µL	85 µL
AST Enzyme	2 µL	-
ODC Enzyme	1 µL	1 µL
Cosubstrate	5 µL	5 µL
Dye reagent	1 µL	1 µL

Transfer 75 µL of Working Reagent into each Sample Plus Standard and Sample well. Transfer 75 µL of Blank Control Reagent each Sample Blank well. Tap plate to mix.

Measurement

1. Incubate the plate protected from light for 30 mins at 37 °C or 60 mins at room temperature.
2. Measure the optical density at 570 nm or fluorescence intensity at λ_{Ex} = 530 nm/ λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔF or ΔOD against the standard concentrations.
3. Determine the slope and calculate Aspartate concentration of samples using the below given equation:

Aspartate (µM) =

$$\frac{R_{Sample} - R_{Blank}}{Slope(\mu M^{-1})} \times DF \quad (\mu M)$$

If an internal standard was used, the sample aspartate concentration is computed as follows:

Aspartate (µM) =

$$\frac{R_{Sample} - R_{Blank}}{R_{Standard} - R_{Sample}} \times 20 \quad (\mu M)$$

Where:

R_{Sample} = OD or fluorescence intensity (F) readings of the Sample
R_{Blank} = OD or fluorescence intensity readings (F) of the Sample Blank
$R_{Standard}$ = OD or fluorescence intensity readings (F) of the Sample Plus Standard
DF = dilution factor.
Conversions: 100 µM aspartate equals 13.2 mg/L, 0.00132% or 13.2 ppm.

Note: The volume of the internal standard is 5× lower than the sample volume; thus, the sample to standard ratio is multiplied by 20 µM.

Note: If the calculated aspartate concentration is > 400 µM for the colorimetric assay, or > 50 µM for the fluorometric assay, dilute sample in purified water and repeat assay. Multiply result by the dilution factor (DF).

Figure 1.
Typical Colorimetric Aspartate Standard Curve

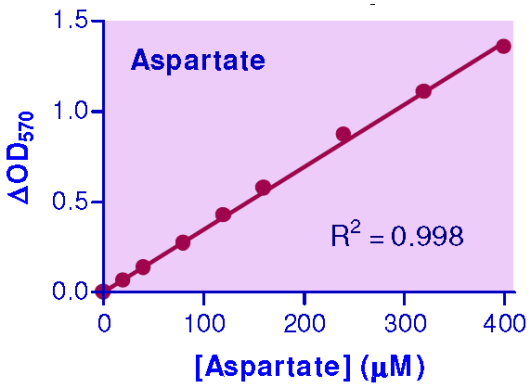
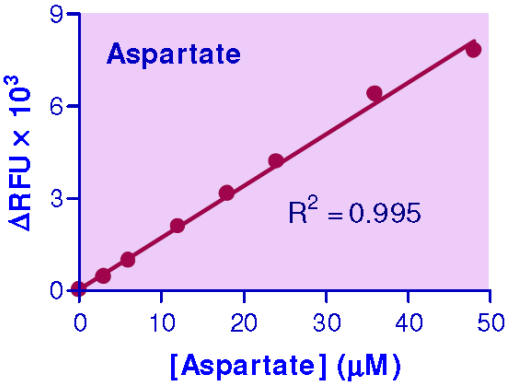


Figure 2.
Typical Fluorometric Aspartate Standard Curve



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