L-Aspartate Assay Kit (Colorimetric/Fluorometric)

LS-K284-100 (100 Tests) • **Store at -20°C**

Introduction

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. LSBio's' 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 λex/em = 530/585 nm is directly proportional to the aspartate concentration in the sample.

Key Features

- Sensitive and accurate. Linear detection range: 2 to 400 µM aspartate for colorimetric assays and 1 to 50 µM for fluorometric assays.

Applications

- Direct Assays: aspartate in plasma, serum, tissue and culture media.
- Drug Discovery/Pharmacology: effects of drugs on aspartate metabolism.

Components

Component	K284-100
	100 Tests
Developer	10 mL
Cosubstrate	600 µL
Dye Reagent	120 µL
AST Enzyme	240 µL
ODC Enzyme	120 µL
Asparate Standard	400 µL

Materials Not Supplied

Pipetting devices, clear or black flat-bottom 96-well plates, plate reader or centrifuge tubes.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
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Assay Procedure

Important: equilibrate Developer to desired assay temperature. The assay requires 30 min when performed at 37°C or 60 min if performed at RT (25°C).

Sample Preparation

Tissue or cell samples (2 x10^6) can be homogenized in 100 µL PBS. Centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay.

Serum and plasma samples should be deproteinated using a 10 kDa spin filter (e.g. Amicon Ultra-0.5). In addition, 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. If planning to measure aspartate in culture media, if possible avoid media with high pyruvate concentrations (e.g. DMEM, L-15, F12, etc.).

Colorimetric Procedure

1. Standards. Dilute the Aspartate Standard to 400 µM by mixing 10 µL 10 mM Standard with 240 µL dH_2O. Next, dilute standards in 1.5-mL centrifuge tubes as described in the table. If assaying culture media with phenol red, dilute the Aspartate Standard in culture media.

No	Premix + dH_2O	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

   Transfer 25 µL of each Standard to separate wells in a clear flat-bottom 96 well plate.

2. Samples. Add 25 µL of each sample to two separate wells in a 96 well plate (each sample requires a Sample Blank). Samples requiring an internal standard, will need three separate reactions: 1) Sample plus Standard, 2) Sample alone and 3) Sample Blank. For the internal standard prepare 500 µL 100 µM aspartate standard by mixing 5 µL 10 mM Standard and 495 µL dH_2O. For the Sample plus Standard well, add 5 µL 100 µM aspartate and 25 µL sample. For the Sample and Sample Blank wells, add 5 µL dH_2O and 25 µL sample.

3. Aspartate Detection. Prepare enough working reagent (WR) for all standards and samples. For each reaction combine the following: 85 µL Developer, 2 µL AST Enzyme, 1 µL ODC Enzyme, 5 µL Cosubstrate and 1 µL Dye Reagent. For the Sample Blanks, prepare a WR without the AST Enzyme. Add 75 µL of the appropriate WR to each Standard and Sample well. Mix well and incubate protected from light for 30 min at 37°C or 60 min at RT.

4. Read OD570nm.

Fluorometric Procedure

For fluorometric assays, the linear detection range is 1 to 50 µM aspartate. Dilute the Standards prepared in Colorimetric Procedure 1:10 in dH_2O. If an internal standard is used, use the same concentration as described in the Colorimetric Procedure (i.e. 5 µL of 100 µM aspartate).

Transfer 25 µL standards and 25 µL samples (2 wells per sample if a standard curve is used; 3 wells per sample if an internal standard is used, see Colorimetric Procedure) into separate wells of a black 96-well plate. Add 75 µL of appropriate Working Reagent (see Colorimetric Procedure) to each well. Tap plate to mix.

Incubate 30 min at 37°C or 60 min at RT and read fluorescence at $\lambda ex/em$ = 530/585 nm.

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Calculations

Subtract the blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the aspartate concentration of the Samples as follows:

$$[\text{Aspartate}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

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

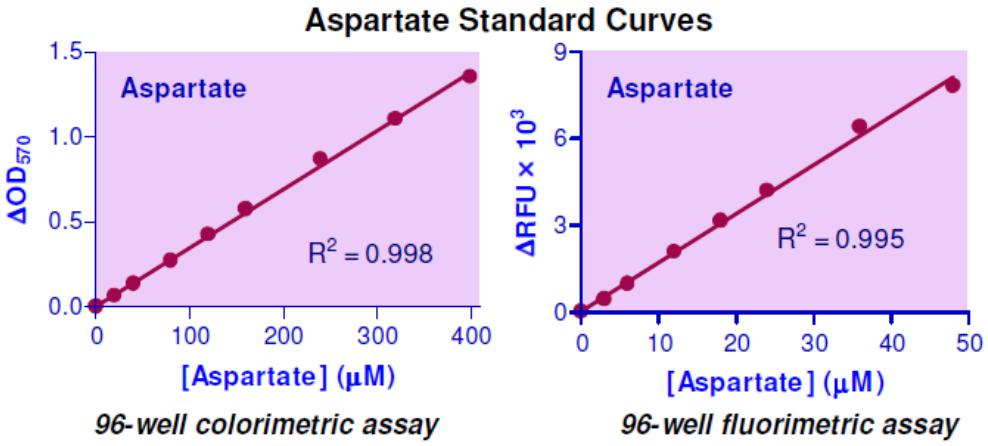
$$[\text{Aspartate}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times 20 \quad (\mu M)$$

where R_{SAMPLE}, R_{BLANK}, and $R_{STANDARD}$ are optical density or fluorescence intensity readings of the Sample, Sample Blank and Sample plus Standard, respectively. n is the sample dilution factor.

Notes: The volume of the internal standard is 5× lower than the sample volume; thus, the sample to standard ratio is multiplied by 20 µM and not 100 µM. If the calculated aspartate concentration is > 400 µM for the colorimetric assay, or > 50 µM for the fluorometric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor n.

Conversions: 100 µM aspartate equals 13.2 mg/L, 0.00132% or 13.2 ppm.

Sample Data



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