EnzyChrom™ L-Aspartate Assay Kit (EASP-100)

Quantitative Colorimetric/Fluorimetric L-Aspartate Determination

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. BioAssay Systems' 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 $\lambda_{ex/em}$ = 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 fluorimetric assays.

APPLICATIONS

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

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Developer:	10 mL	**AST Enzyme:**	240 µL
Dye Reagent:	120 µL	**Cosubstrate:**	600 µL
ODC Enzyme:	120 µL	**Aspartate Standard**:	400 µL

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.
Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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 OD_{570nm}.

Fluorimetric Procedure

For fluorimetric 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.

CALCULATION

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:

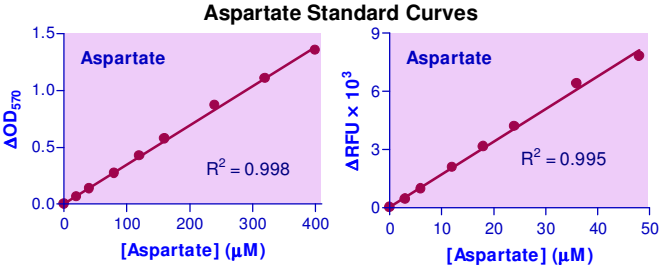
$$[\text{Aspartate}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times 20 \quad (\mu M)$$

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 fluorimetric 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.

Aspartate Standard Curves



96-well colorimetric assay ***96-well fluorimetric assay***

MATERIALS REQUIRED, BUT NOT PROVIDED

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

LITERATURE

1. Baetz, AL. et al (1975). Developmental Changes of Free Amino Acids in Bovine Fetal Fluids with Gestational Age and the Interrelationships between the Amino Acid Concentrations in the Fluid Compartments. J.Reprod. Fert. 44:437-44.
2. Graham, LT and Aprison, MH (1966). Fluorometric determination of aspartate, glutamate, and γ-aminobutyrate in nerve tissue using enzymic method. Anal. Biochem.15: 487-97.
3. Parvin, R et al (1980). Convenient rapid determination of picomole amounts of oxaloacetate and aspartate. Anal. Biochem.104: 296-9.

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