Product Information

Aspartate Colorimetric/Fluorometric Assay Kit

I. Kit Contents:

Components	K2082-100 100 assays	Cap Color	Part Number
Aspartate Assay Buffer	25 ml	WM	K2082-C-1
Probe (DMSO solution)	0.2 ml	Red	K2082-C-2
Serum Clean Up Mix	lyophilized	Blue	K2082-C-3
Aspartate Enzyme Mix	lyophilized	Green	K2082-C-4
Conversion Mix	lyophilized	Purple	K2082-C-5
Aspartate Standard (100 mM)	0.1 ml	Yellow	K2082-C-6

II. Introduction:

Aspartate (Asp) is an α-amino acid. L-Aspartic acid is one of the 23 proteinogenic amino acids and serves as a precursor to four essential amino acids (Met, Thr, Ile and Lys). Aspartate is a metabolite in the urea cycle and is involved in gluconeogenesis and transports reducing equivalents between the mitochondria and the cytosol via the malate-aspartate shuttle. Also, aspartate is an excitotoxin and serves as an excitatory neurotransmitter in the brain through the stimulation of NMDA receptors.

The Aspartate Colorimetric/Fluorometric Assay Kit provides a sensitive, simple and convenient way for detection of aspartate in a variety of samples based on colorimetric and fluorometric method. In the assay, aspartate is converted to pyruvate which is oxidized with the conversion of a probe into a fluorescent (Ex/Em 535/587 nm) and highly colored (570 nm) species proportional to the amount of aspartate in samples. Aspartate can be quantified in the range between 0.1 – 10 nmoles/well (2 - 200 µM).

III. Reagent Preparation, Storage and Handling:

Store the kit at -20°C prior to use. Read the entire protocol beafor performing the assay.

Aspartate Probe: Ready to use as supplied. Warm the probe to room temperature to melt the DMSO prior to use.

Serum Clean Up Mix, Aspartate Enzyme Mix, Conversion Mix: Add 220 µl of Aspartate Buffer to each vial respectively and dissolve completely prior to use. These can be kept for up to a week after reconstitution. If use over a longer period is anticipated, they should be aliquoted and stored at -20°C.

IV. Assay Protocol:

1. Standard Curve Preparation:

Colorimetric Assay: Dilute the Aspartate Standard to 1.0 mM by adding 10 µl of the 100 mM Aspartate Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Aspartate Standard.

Fluorometric Assay: Dilute the Aspartate standard to 1 mM as in the colorimetric Assay. Dilute further another 10X by taking 100 µl of the standard and adding 900µl of dH_2O mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Aspartate Standard.

2. Sample Preparation:

Cell extracts can be used directly in the assay. Serum samples require pretreatment to remove interfering substances: Add 2 µl of the Serum Clean Up Mix to 100 µl serum and incubate 30 min at room temperature. Treated serum samples should be deproteinized by centrifuging 10 min with a 10 kDa spin filter Filtrate (1 - 30 µl) can be used directly in the assay. Adjust all well volumes to 50 µl with Assay Buffer. Due to the relatively low levels of aspartate in serum, use of the fluorometric assay is strongly recommended.

3. Reaction:

Prepare 50 µl of reaction mix for each standard and sample well to be measured. The reaction mix consists of:

	Reaction Mix	Background Control
Aspartate Enzyme Mix	2 µl	---
Conversion Mix	2 µl	2 µl
Probe	2 µl	2 µl
Aspartate Buffer	44 µl	46 µl

In order to reduce background in the fluorometric assay, reduce the amount of probe per well to 0.5 µl per well

Samples may contain relatively high levels of pyruvate which will increase background. In that case a background control is needed to correct for pyruvate.

4. Incubate: For 30 min at room temperature

5. Read: Measure OD at 570 nm or fluorescence at Ex/Em 535 nm/587 nm in a microplate reader.

6. Calculation:

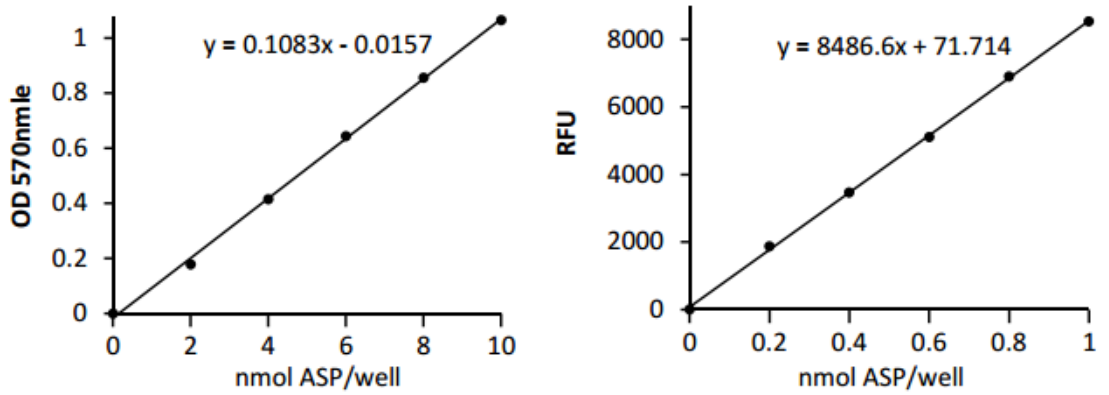
Correct background by subtracting the value derived from the 0 Standard from all readings (The background reading can be significant and must be subtracted). Plot the Standard curve. Read sample concentrations from the standard curve:

C = Sa/Sv nmol/µl or mM,

Where Sa is the sample amount (in nmol) from standard curve.

Sv is the sample volume (µl) added into the wells.

Aspartate MW: 65.384 g/mol.



Colorimetric and Fluorometric standard curves obtained following this protocol.

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com