amsbio

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For research use only

Aspartate Assay Kit

(Catalog #K552-100; 100 assays; Store kit at -20°C)

I. Introduction: Aspartic acid is one of the 20 proteinogenic amino acids. In addition to its role in protein synthesis, it is a precursor to several other amino acids including four which are essential, participates in the urea cycle and gluconeogenesis and is an excitatory neuro-transmitter similar to glutamate. Aspartate can be used to transport reducing equivalents between the cytosol and mitochondria through the aspartate-malate shuttle. BioVision's Aspartate Assay Kit is a simple convenient format to measure aspartate in a variety of samples. In the assay aspartate is converted to pyruvate which is oxidized with the conversion of our probe into a highly colored (OD 570) and fluorescent (Ex/Em 535/587) species proportional to the amount of aspartate present. Aspartate can be quantified in the range between 0.1 – 10 nmoles. Serum aspartate normal range is 0 (undetectable) -25 nmol/ml.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Aspartate Assay Buffer	25 ml	WM	K552-100-1
Probe (DMSO solution)	0.2 ml	Red	K552-100-2A
Serum Clean Up Mix	lyophilized	Blue	K552-100-3
Aspartate Enzyme Mix	lyophilized	Green	K552-100-4
Conversion Mix	lyophilized	Purple	K552-100-5
Aspartate Standard (100 mM)	0.1 ml	Yellow	K552-100-6

III. Reagent Preparation, Storage and Handling:

Store the kit at -20°C prior to use. **READ THE ENTIRE PROTOCOL BEFORE PERFORMING THE ASSAY.**

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

Sample Clean Up Mix, Aspartate Enzyme Mix, Substrate 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 (BioVision #1997-25-**Not included**). 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 570nm or fluorescence at Ex/Em 535nm/587nm in a microplate reader.
6. **Calculation:**

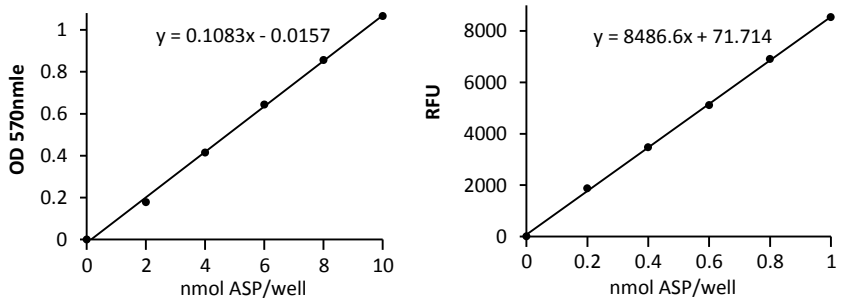
Correct background by subtracting the value derived from the 0 Standard from all readings (The background reading can be significant and must be subtracted). Plot the Standard curve. Read sample concentrations from the standard curve:

$C = S_a/S_v$ nmol/µl or mM,

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

S_v is the sample volume (µl) added into the wells

Aspartate MW: 65.384 g/mol



Colorimetric and Fluorometric standard curves obtained following this protocol.

RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit
Ascorbic Acid Quantification Kit
Glucose Assay Kit
Uric Acid Assay Kit
Pyruvate Assay Kit
Triglyceride Assay Kit
Choline/Acetylcholine Quantification Kit
Antioxidant Capacity (TAC) Assay Kit
L-amino Acid Assay Kit
Ethanol Assay Kit
ADP/ATP Ratio Assay Kit
Glutathione Detection Kits
Fatty Acid Assay Kit
Alanine Assay Kit
Lactate Assay Kit/ II
Phosphate Assay Kit
Hemin Assay Kit
Glycogen Assay Kit
Nitric Oxide Assay Kits
Urea Assay Kit

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