

## 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 neurotransmitter 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<sub>2</sub>O, 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<sub>2</sub>O, 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:**

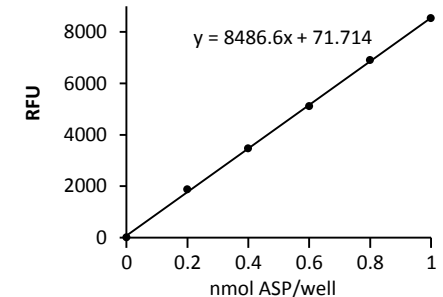
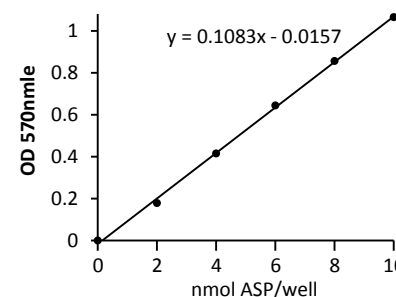
Correct background by subtracting the value derived from the 0 Standard from all readings (The background reading can be significant and must be subtracted). Plot the Standard curve. Read sample concentrations from the standard curve:

$$C = S_a/S_v \text{ nmol/}\mu\text{l or mM,}$$

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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