

Aspartate Colorimetric/Fluorometric 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 (in DMSO)	0.2 ml	Red	K552-100-2A
Serum Clean Up Mix	1 vial	Blue	K552-100-3
Aspartate Enzyme Mix	1 vial	Green	K552-100-4
Conversion Mix	1 vial	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.**

Probe: Ready to use as supplied. Warm the vial to room temperature (RT) to melt the DMSO prior to use.

Serum Clean Up Mix, Aspartate Enzyme Mix, Conversion Mix: Add 220 µl of Aspartate Assay 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 dH₂O, 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 dH₂O, 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 RT

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

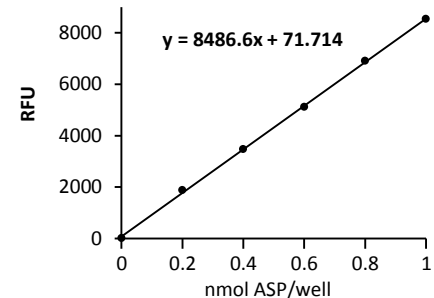
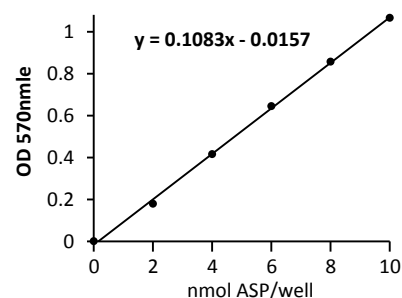
6. Calculation: Correct background by subtracting the value derived from the 0 Standard from all readings (The background reading can be significant and must be subtracted). Plot the Standard Curve. Read sample concentrations from the Standard Curve:

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

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: 133.11 g/mol



Colorimetric and Fluorometric Standard Curves obtained following this protocol

RELATED PRODUCTS:

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| Ascorbic Acid Quantification Kit | Glutathione Detection Kits |
| Glucose Assay Kit | Fatty Acid Assay Kit |
| Uric Acid Assay Kit | Alanine Assay Kit |
| Pyruvate Assay Kit | Lactate Assay Kit/ II |
| Triglyceride Assay Kit | Phosphate Assay Kit |
| Choline/Acetylcholine Quantification Kit | Hemin Assay Kit |
| Antioxidant Capacity (TAC) Assay Kit | Glycogen Assay Kit |
| L-amino Acid Assay Kit | Nitric Oxide Assay Kits |
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