ab102512

Aspartate Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Aspartate levels in various samples

This product is for research use only and is not intended for diagnostic use.

Version 6b, Last Updated 7 July 2025

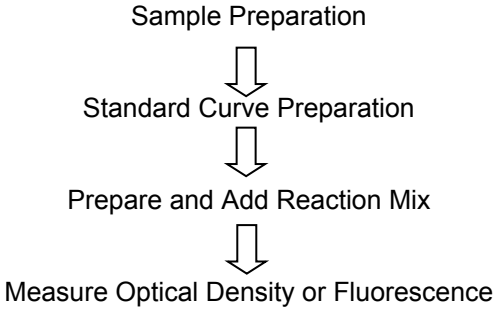
Table of Contents

1. Overview

L-Aspartic acid (Asp) is one of the 20 proteinogenic amino acids as building block for protein. Although a non-essential amino acid in mammals, it is a precursor to several other amino acids including four essential amino acids (Met, Thr, Ile and Lys). Aspartate is a metabolite in the urea cycle, participates in gluconeogenesis and transports reducing equivalents between the cytosol and the mitochondria via the malate-aspartate shuttle. Aspartate also stimulates NMDA receptors (not as strongly as glutamate) and hence serves as an excitatory neurotransmitter in the brain and is an excitotoxin.

Abcam's Aspartate Assay Kit provides a simple, convenient assay to measure aspartate in a variety of samples. In the assay, aspartate is converted to pyruvate which is oxidized with the conversion of a probe into a highly colored (570 nm) and fluorescent (Ex/Em 535/587 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).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 4	25 mL
OxiRed™ Probe	0.2 mL
Sample Clean-Up Mix I	1 vial
Aspartate Enzyme Mix	1 vial
Converter Mix J	1 vial
Aspartate Standard	0.1 mL

* Store kit at -20°C. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and Aspartate Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Probe (DMSO solution). Converter Mix J was previously labelled as Converter Enzyme XII and Conversion Mix (Lyophilized). The composition has not changed.

OXIRED™ PROBE: Ready to use as supplied. Warm the probe to room temperature to melt the DMSO prior to use

SAMPLE CLEAN-UP MIX I, ASPARTATE ENZYME MIX, CONVERTER MIX J: 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.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Cell extracts can be used directly in the assay.

Serum samples require pre-treatment to remove interfering substances:

a) Add 2 µl of the Sample Clean-Up Mix I to 100 µl serum and incubate 30 min at room temperature.

b) Treated serum samples should be deproteinized by centrifuging 10 min with a 10 kDa spin filter (**ab93349**). Filtrate (1-30 µl) can be used directly in the assay.

Adjust all well volumes to 50 µl with Assay Buffer 4.

Note:

Due to the relatively low levels of aspartate in serum, use of the fluorometric assay is strongly recommended.

2. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute the Aspartate Standard to 1.0 mM by adding 10 µl of the 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 4 to generate 0, 2, 4, 6, 8, 10 nmol/well of the Aspartate Standard.

b. For the 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 4 to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Aspartate Standard.

3. Reaction Mix:

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

	Sample	**Background Control****
Aspartate Enzyme Mix	2 µl	--
Converter Mix J	2 µl	2 µl
OxiRed™ Probe*	2 µl	2 µl
Assay Buffer 4	44 µl	46 µl

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

****Note:** 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.

5. Data Analysis

Correct background by subtracting the value derived from the zero 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:

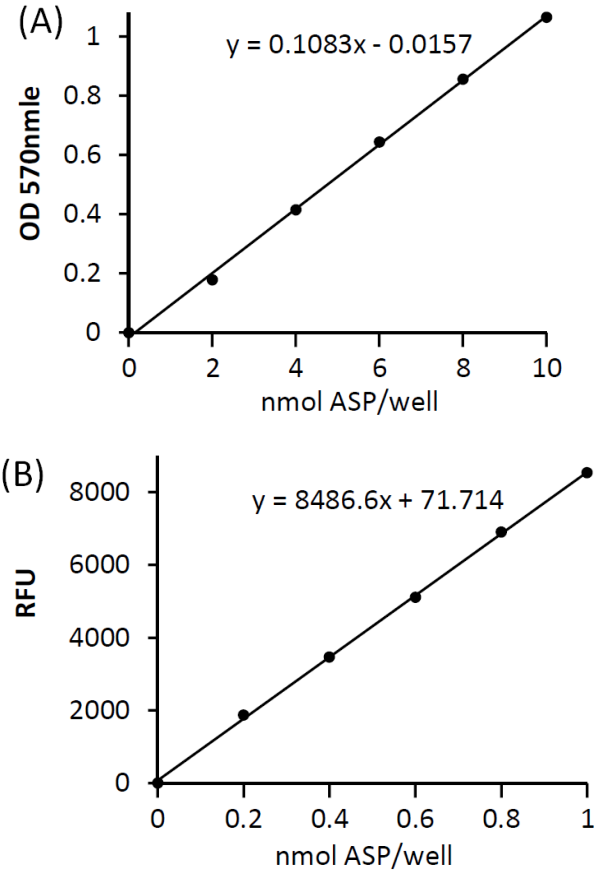
Concentration = Sa / Sv (nmol/µl or mM)

Where

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

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

Aspartate MW: 133.11 g/mol



Aspartate Colorimetric (A) and Fluorometric (B) standard curves generated using this kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)