Technical Bulletin

Aspartate Assay Kit

Catalogue number MAK095

Product Description

Aspartate, the carboxylate anion of aspartic acid, is an acidic, non-essential amino acid involved in protein synthesis and multiple other cellular biochemical pathways. Aspartate contributes to nucleotide synthesis via the synthesis of the precursor inosine monophosphate. In the urea cycle, aspartate is a key metabolite, donating a nitrogen group towards the formation of urea. Aspartate is also critical for oxidative phosphorylation as part of the aspartate-malate shuttle, which transfers reducing equivalents across the mitochondrial membrane.

The Aspartate Assay Kit is suitable for aspartate detection in cell and tissue culture supernatants, urine, plasma, serum, and other biological samples. Aspartate concentration is determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/ fluorometric (λ_{Ex} = 535/λ_{Em} = 587 nm) product, proportional to the aspartate present. Typical detection ranges for this kit are 2–10 nmole (colorimetric) and 0.2–1 nmole (fluorometric).

Components

The kit is sufficient for 100 assays in 96-well plates.

- Aspartate Assay Buffer — 25 mL
  Catalogue Number MAK095A
- Probe, in DMSO — 0.2 mL
  Catalogue Number MAK095B
- Serum Clean Up Mix — 1 vl
  Catalogue Number MAK095C
- Aspartate Enzyme Mix — 1 vl
  Catalogue Number MAK095D
- Conversion Mix — 1 vl
  Catalogue Number MAK095E
- Aspartate Standard, 100 mM — 0.1 mL
  Catalogue Number MAK095F

Reagents and Equipment Required but Not Provided.

- 96-well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays. Cell culture or tissue culture treated plates are not recommended.
- Fluorescence or spectrophotometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filters

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Aspartate Assay Buffer – Allow buffer to come to room temperature before use.

Probe – Warm to room temperature to melt frozen solution prior to use. Store protected from light and moisture at -20 °C. Upon thawing, the Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the colorimetric Probe Solution 4-fold with Aspartate Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Serum Clean Up Mix, Aspartate Enzyme Mix, and Conversion Mix – Reconstitute each in 220 µL of Aspartate Assay Buffer. Mix well by pipetting, then aliquot each and store at -20 °C. Keep cold while in use and protect from light. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice. Storage at -20 °C, protected from light, is recommended.

Procedure

All Samples and Standards should be run in duplicate.

Aspartate Standards for Colorimetric Detection

Dilute 10 µL of the 100 mM (100 nmole/µL) Aspartate Standard Solution with 990 µL of water to prepare a 1 mM (1 nmole/µL) Aspartate Standard. Prepare Aspartate Standards for colorimetric assay according to Table 1. Mix well.

Table 1.
Preparation of Aspartate Standards for colorimetric assay

Well	1 mM Aspartate Standard	Aspartate Assay Buffer	Aspartate (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Aspartate Standards Fluorometric Detection

Prepare a 1 mM Aspartate Standard solution as for the colorimetric assay. Dilute 100 µL of the 1 mM Aspartate Standard with 900 µL of water to make a 0.1 mM (0.1 nmole/µL) Aspartate Standard. Prepare Aspartate Standards for fluorometric assay according to Table 2. Mix well.

Table 2.

Preparation of Aspartate Standards for fluorometric assay

Well	0.1 mM Aspartate Standard	Aspartate Assay Buffer	Aspartate (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	0.2
3	4 µL	46 µL	0.4
4	6 µL	44 µL	0.6
5	8 µL	42 µL	0.8
6	10 µL	40 µL	1.0

Sample Preparation

Both the colorimetric and fluorometric assays require 50 µL of Sample for each reaction (well).

Tissue or cells (1×10^6) can be homogenized in 100 µL of the Aspartate Assay Buffer. Centrifuge the Samples at 13,000 × g for 10 minutes to remove insoluble material. Bring Samples to a final volume of 50 µL with Aspartate Assay Buffer.

Serum Samples should be pretreated with the Serum Clean Up Mix to remove interfering substances. Add 2 µL of the Serum Clean Up Mix to 100 µL of serum and incubate for 30 minutes at room temperature. Treated Samples should be deproteinized before use in assay with a 10 kDa MWCO spin filter. 1–30 µL of deproteinized serum Samples can be directly diluted to a final volume of 50 µL with the Aspartate Assay Buffer.

Note: Due to relatively low levels of aspartate in serum, it is strongly recommended to use the fluorometric assay, which is typically 10-fold more sensitive than the colorimetric assay.

For unknown Samples, it is suggested to test several Sample dilutions to ensure the readings are within the linear range of the Standard curve.

Pyruvate present in the Sample can generate background. To control for pyruvate background, include a blank Sample for each Sample by omitting the Aspartate Enzyme Mix in the Reaction Mix.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 3. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 3.

Reaction Mixture

Reagent	Sample Blank	Samples and Standards
Aspartate Assay Buffer	46 µL	44 µL
Aspartate Enzyme Mix	-	2 µL
Conversion Mix	2 µL	2 µL
Probe	2 µL	2 µL

2. Add 50 µl of the appropriate Reaction Mix to each of the blank, Standard, and test wells. Mix well using a horizontal shaker or by pipetting.
3. Incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.
4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (RFU) at λ_{Ex} = 535/λ_{Em} = 587 nm.

Results

Calculations

1. The background for the assays is the value obtained for the 0 (blank) Aspartate Standard. Correct for the background by subtracting the blank value (A_{570} or RFU) from all readings. Background values can be significant and must be subtracted from all readings.
2. Use the corrected values obtained from the appropriate Aspartate Standards to plot a Standard curve.

   Note: A new Standard curve must be set up each time the assay is run.
3. Subtract the Sample Blank value (A_{570} or RFU) from the Sample readings to obtain the corrected measurement.
4. Using the corrected Sample A_{570} or RFU measurement, the amount of aspartate present in the Sample may be determined from the Standard curve.

Concentration of Aspartate

$$S_A/V = C$$

S_A = Amount of aspartate in unknown Sample (nmole) from Standard curve
V = Sample volume (µl) added into the wells
C = Concentration of aspartate in Sample
Aspartate molecular weight: 133.11 g/mole.

Sample Calculation

Amount of aspartate (S_A) = 5.84 nmole
Sample volume (V) = 50 µL
Concentration of aspartate in Sample:
5.84 nmole/50 µL = 0.1168 nmole/µL
0.1168 nmole/µL × 133.11 ng/nmole = 15.55 ng/µL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96-well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of older kit or improperly stored reagents	Check the receipt date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak095pis Rev 09/23