Technical Bulletin

D-Amino Acid Assay Kit

Catalogue number MAK537

Product Description

D-amino acids are not as widespread as their enantiomeric counterparts in proteins, but they can be found in organisms ranging from bacteria (cell walls and antibiotics) to mammals (central nervous systems). The presence of D-amino acids in food is also of considerable interest. Racemization of L-amino acids during food processing may affect food quality and nutritional value.

The D-amino Acid Assay Kit uses an enzyme catalyzed oxidation of D-amino acids to convert a dye into a colored and fluorescent form. The absorbance at 570 nm or fluorescence intensity at $\lambda_{ex}/\lambda_{em}$ = 530/585 nm.

The linear detection range of the kit is 0.86 to 500 µM for the colorimetric assay and 0.18 to 50 µM for the fluorometric assay. The kit is suitable for D-amino acid determination in biological samples such as tissue and food and beverage samples such as milk.

Components

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

- Assay Buffer 12 mL
  Catalogue Number MAK537A
- HRP Enzyme 120 µL
  Catalogue Number MAK537B
- DAA Enzyme 120 µL
  Catalogue Number MAK537C
- Dye Reagent 120 µL
  Catalogue Number MAK537D
- Standard (2 mM) 500 µL
  Catalogue Number MAK537E

Reagents and Equipment Required but Not Provided

1. Pipetting devices and accessories (example., multichannel pipettor)
2. Spectrophotometric multiwell plate reader.
3. Clear flat-bottom 96-well plates or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
4. Dounce Tissue Grinder Set (Catalogue Number D9063 or equivalent)
5. 1.5 mL microcentrifuge tubes.

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.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate all reagents to room temperature.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Tissue Samples

1. Homogenize 20-100 mg of tissue in 200-1000 µL purified water.
2. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
3. Remove supernatant for assay.

Milk Samples

Often require a 2X dilution in Assay Buffer.

Colorimetric Standard Curve Reaction

1. Prepare 200 µL of 500 µM Standard by mixing 50 µL of the Standard (2 mM) with 150 µL of purified water.
2. Prepare standards in 1.5 mL centrifuge tubes with purified water according to Table 1.

Table 1.
Preparation of Colorimetric Standards

Well No.	500 µM Standard	Purified Water	D-Amino Acid (µM)
1	100 µL	0 µL	500
2	60 µL	40 µL	300
3	30 µL	70 µL	150
4	0 µL	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 µL of the standards from Colorimetric Procedure with 90 µL of purified according to Table 2.

Table 2.
Preparation of Fluorometric Standards

Well	Colorimetric Standard	Purified Water	D-Amino Acid (µM)
1	10 µL of 500 µM Std	90 µL	50
2	10 µL of 300 µM Std	90 µL	30
3	10 µL of 150 µM Std	90 µL	15
4	-	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a black 96 well plate.

Working Reagent Preparation

For each well, prepare 88 µL of Working Reagent to Table 3.

Table 3.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	85 µL
HRP Enzyme	1 µL
DAA Enzyme	1 µL
Dye Reagent	1 µL

Assay Reaction

1. Add 80 µL of the working reagent to each well. Tap plate to mix.
2. Incubate protected from light for 60 minutes at room temperature.
3. Measure the optical density (OD) at 570 nm for colorimetric assay or measure fluorescence at λ_{em} = 585 nm/λ_{ex} = 530 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against the standard concentrations.
3. Determine the slope and calculate the D Amino Acid concentration of samples using the below equation:

$$\text{D-Amino Acid } (\mu M) = \left(\frac{R_{Sample}-R_{Blank}}{Slope(\mu M^{-1})} \times DF\right)$$

Where:

R_{Sample} = Fluorescence intensity (F) or OD reading of Sample

R_{Blank} = Fluorescence intensity (F) or OD reading of Sample Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Figure 1

Typical Colorimetric Standard Curve

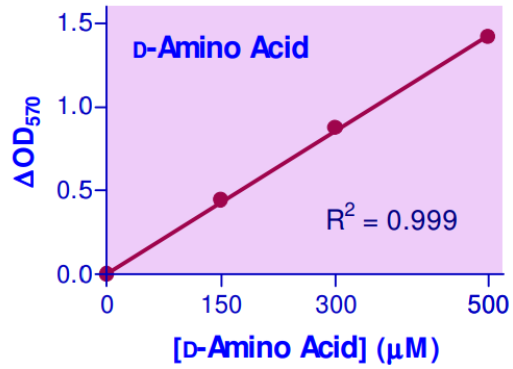
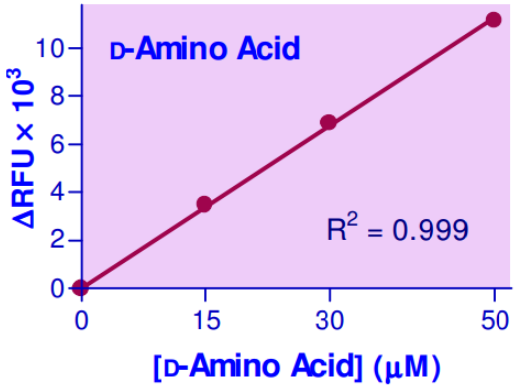


Figure 2

Typical Fluorometric Standard Curve



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