D-Amino Acid Assay Kit (BA0237)

Product Datasheet | SKU BA0237

Description

A quantitative colorimetric and fluorimetric assay for D-amino acids using an enzyme-catalysed oxidation of D-amino acids to convert a dye into a coloured and fluorescent form. The absorbance at 570 nm or fluorescence intensity at λex/em = 530/585 nm is directly proportional to the D-amino acid concentration in the sample.

Specifications

Detection Method	Colorimetric (OD570 nm) / Fluorimetric (λex/em = 530/585 nm)
Detection Range	0.86 to 500 µM (colorimetric) and 0.18 to 50 µM (fluorimetric) for 60 min reaction
Sample Type	Tissue, milk and other biological preparations
Species Reactivity	All
Assay Time	60 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped on ice. Store all components at -20°C upon receiving.
Shelf Life	6 months after receipt.
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	12 mL	-20°C
HRP Enzyme	120 µL	-20°C
DAA Enzyme	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard	500 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0237	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Sample preparation: tissue samples can be prepared by homogenising 20-100 mg in 200-1000 µL dH2O, centrifuging at 10,000 × g for 15 min at 4°C and removing supernatant for assay. Milk samples often require at least 2× dilution in Assay Buffer.
2. Reagent preparation: equilibrate all reagents to room temperature; briefly centrifuge tubes before opening.
3. Colorimetric standards: prepare 200 µL of 500 µM Premix by mixing 50 µL Standard (2 mM) and 150 µL dH2O; dilute standards in 1.5-mL tubes (500, 300, 150, 0 µM) per the table. Transfer 20 µL Standards into separate wells of a clear flat-bottom 96-well plate.
4. Transfer 20 µL of each sample into separate wells.
5. Prepare enough Working Reagent (WR) for all assay wells by mixing, per well, 85 µL Assay Buffer, 1 µL DAA Enzyme, 1 µL HRP Enzyme and 1 µL Dye Reagent.

6. Add 80 µL WR to each well. Tap plate briefly to mix. Incubate at room temperature for 60 min. Measure OD570nm.
7. Fluorimetric procedure: dilute the colorimetric standards 1:10 in dH2O; transfer 20 µL standards and 20 µL samples into separate wells of a black flat-bottom 96-well plate. Add 80 µL WR to each well, tap to mix, incubate protected from light for 60 min at room temperature and measure fluorescence at λex/em = 530/585 nm.

Colorimetric Standards

No.	Premix	H2O	Standard (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

Calculation

Subtract the blank value (water, standard #4) from the standard values and plot the adjusted values against standard concentrations to determine the slope. [D-Amino Acid] = [(R_Sample – R_Blank) / Slope (μM^{-1})] × n (µM), where R_Sample and R_Blank are the OD or fluorescence values of the Sample and H2O Blank, respectively, and n is the sample dilution factor.

Notes

- This assay is based on an enzyme-catalysed kinetic reaction; addition of Working Reagent should be quick and mixing brief but thorough.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.