# D-Amino Acid Microplate Assay Kit

# User Manual

## Catalog # CAK1297

(Version 1.1A)

Detection and Quantification of D-Amino Acid content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

## I. INTRODUCTION

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.

D-Amino Acid Microplate Assay Kit provides a simple and direct procedure for measuring D-Amino Acid content in a variety of samples. D-amino acid assay uses an enzyme-catalyzed oxidation of D-amino acids to convert a dye into a colored form. The optical density of the colored product at 570nm is directly proportional to the D-Amino Acid concentration in the sample.

## II. KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30 ml x 4 | 4 °C |
| Reaction Buffer | 10 ml x 1 | 4 °C |
| Enzyme | Powder x 1 | -20 °C |
| Dye Reagent | Powder x 1 | -20 °C, keep in dark |
| Standard | Powder x 1 | 4 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

**Note:**

**Standard**: add 1 ml distilled water to dissolve before use, mix; then add 0.5 ml into 0.5 ml distilled water, the concentration will be 50 mmol/L; store at -20 °C for 1 month after reconstitution.

**Enzyme**: add 1 ml Reaction Buffer to dissolve before use, mix; store at -80 °C for 1 month after reconstitution.

**Dye Reagent**: add 10 ml distilled water to dissolve before use, mix, store at 4 °C for 1 week after reconstitution.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

## IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for $5 \times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

## V. ASSAY PROCEDURE

Add following reagents into the microplate:

| Reagent | Standard | Blank | Sample |
|---|---|---|---|
| Reaction Buffer | 80 µl | 80 µl | 80 µl |
| Enzyme | 10 µl | 10 µl | 10 µl |
| Standard | 10 µl | -- | -- |
| Distilled water | -- | 10 µl | -- |
| Sample | -- | -- | 10 µl |
| Dye Reagent | 100 µl | 100 µl | 100 µl |
| Mix, put it in the oven, incubate at 37 °C for 10 minutes, measured at 570 nm and record the absorbance. | | | |

**Note:**

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

1. According to the protein concentration of sample

$$DAA\ (\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$DAA\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$DAA\ (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$DAA\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 50 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the standard concentration, 50 mmol/L = 50 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × $10^4$;

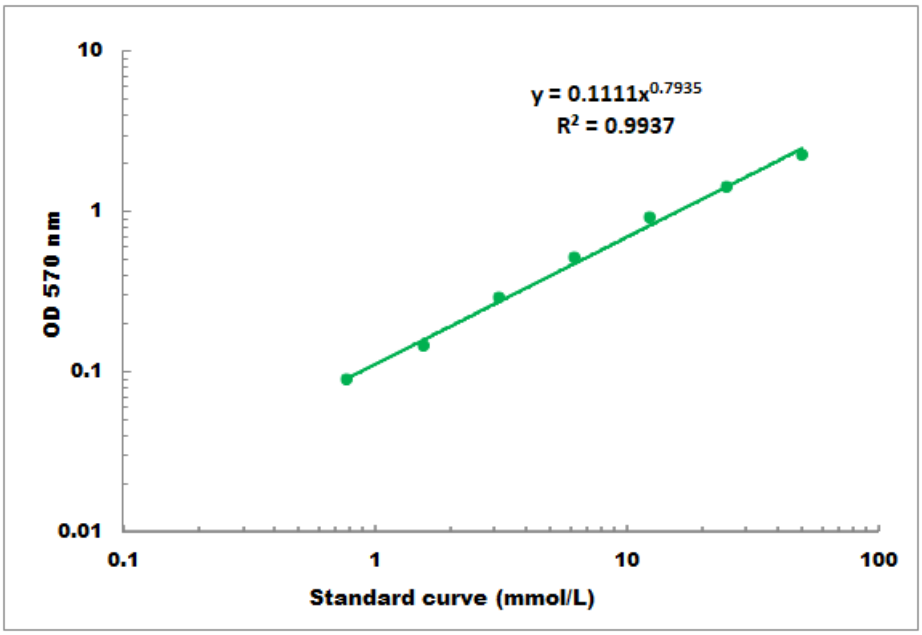
$V_{Sample}$: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.01 ml;

$V_{Assay}$: the volume of Assay buffer, 1 ml.

## VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.




Detection Range: 0.5 mmol/L - 50 mmol/L

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES