D-Serine Colorimetric Assay Kit

Introduction

D-form amino acids have long been known as components of bacterial cell wall peptidoglycan layers. More recent developments in analytical technologies have demonstrated that D-amino acids are also present in mammals and display specific and important physiological activities. Particularly high levels of D-serine levels are present in brain tissue where D-serine functions as an important co-agonist of N-methyl-D-aspartate (NMDA) receptors (NMDR), involved in regulating higher brain function such as memory and learning. D-serine is suggested to play a role in neurodegeneration associated with diseases such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). High levels of free D-serine can also be found in human urine although its significance is unclear.

D-serine is commonly assayed by HPLC or GC following conversion of D-serine to diastereomer derivatives. Such methods are time consuming, require expensive instrumentation, and are not suitable for processing large numbers of samples. The D-Serine Colorimetric Assay Kit employs the D-form specific enzyme D-serine dehydratase from Saccharomyces cerevisiae （DsdSC） enabling the quantitation of D-serine by spectrophotometric measurement.

< Advantages >

1. Enzymatic reaction with colorimetric detection for reading on standard UV/VIS absorbance microplate readers (340 nm).
2. Suitable for large numbers of samples.
3. Quantitative for D-serine detection. Detection range : 0.01 mM-1mM

< Principle of Assay >

DsdSC catalyzes the conversion of D-serine to pyruvate and ammonia. In the presence of lactate dehydrogenase (LDH), pyruvate is reduced to lactate with the concomitant oxidation of NADH to NAD. The reaction can be monitored by measuring the decrease in absorbance at 340nm due to the oxidation of NADH. The D-serine concentration in unknown samples is determined by comparison with a standard curve.

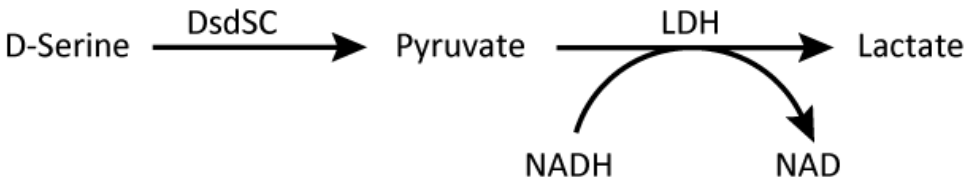

Figure 1: Principle of D-Serine Colorimetric Assay Detection

< Sample type >

• Urine

I. Kit Components

Reagents for 50 reactions are supplied.
Unopened kit: Stable at -20°C until expiration date printed on the label.

Reagent	Volume	Quantity	Storage
Assay Buffer	11 mL	2 vials	Opened reagents are possible to store at -20°C. Protect from light
LDH Diluent Buffer	3 mL	2 vials	
10mM D-Serine Solution	2 mL	2 vials	
NADH Solution ***Green Cap Tube**	200 µL	2 vials	
DsdSC Solution ***Red Cap Tube**	110 µL	1 vial	
LDH Stock Solution ***Blue Cap Tube**	220 µ	1 vial	
96-wellplate	96-well	1 plate	Possible to store at Room Temperature

* Required measuring apparatus is a microplate reader at 340nm wavelength with an optical density range of 0-4 OD.
* DsdSC : D-Serine dehydratase from *Saccharomyces cerevisiae*.

II. Preparation of Urine Sample

Centrifuge urine sample at 4°C at 10,000 x g for 5 minutes. Transfer supernatant to a new tube. The supernatant is used as unknown sample for **IV. Measurement method.** Urine samples do not need to be diluted for assay. Store urine samples on ice until assay or freeze at -20°C and protect from light.

III. Preparation of Reaction Solution

- Thaw all reagents completely and mix by vortexing gently.
- Keep thawed reagents on ice until use.
- Protect from light.

Preparation of D-serine standard (80 µL standard solution used per reaction)

- Prepare a D-serine standard dilution series (0 to 1 mM) by diluting the 10mM D-serine stock solution in ultrapure water.
- Prepare shortly before use.
- Keep D-serine standards on ice until use.

Preparation of Reaction Mix A (160 µL Reaction Mix A used per reaction)

- Prepare Reaction Mix A for 10 reactions as shown in Table 1. Mix thoroughly by vortexing gently.
- Prepare shortly before use.
- Keep Reaction Mix A on ice until use.
- Protect from light.

Table 1

Reagent	Volume per 10 reactions (Includes overage to compensate for volume loss)
Assay Buffer	2000 µL
NADH Solution ***Green Cap Tube**	30 µL
DsdSC Solution ***Red Cap Tube**	20 µL

* Before opening tube, centrifuge briefly to spin down any content on tube wall.

Preparation of Reaction Mix B (160 µL Reaction Mix B used per reaction)
• Prepare Reaction Mix B for 10 reactions as shown in Table 2. Mix thoroughly by vortexing gently.
• Prepare shortly before use.
• Keep Reaction Mix B on ice until use.
• Protect from light.

Table 2

Reagent	Volume per 10 reactions (Includes overage to compensate for volume loss)
Assay Buffer	2000 µL
NADH Solution ***Green Cap Tube**	30 µL

* Before opening tube, centrifuge briefly to spin down any content on tube wall.

Preparation of LDH Solution (10 µL LDH Solution used per reaction)
Note : Prepare just before use. See **IV.(2)** below.
• Prepare LDH Solution for 10 reactions as shown in Table 3. Mix thoroughly by vortexing gently.
• Keep LDH Solution on ice until use.
• Protect from light.

Table 3

Reagent	Volume per 10 reactions (Includes overage to compensate for volume loss)
LDH diluent buffer	500 µL
LDH stock solution ***Blue Cap Tube**	20 µL

* Before opening tube, centrifuge briefly to spin down any content on tube wall.

IV. Measurement method

Read the following precautions prior to measurement.
• A freshly prepared standard curve should be used each time when the assay is performed.
• Insure that no solutions stick to the side of assay wells. Flash centrifuge as required. Measurement results may be severely compromised if care is not taken.
• Before reading absorbance values, and after adding reaction components, mix reaction components thoroughly using a microplate mixer.

Assay is performed as follows:
Quantity of solutions to be added per well of a 96-well plate are given here.
(1) ***Standard well*** : Add 80µL of **D-Serine Standard per well**. Add 160 µL of **Reaction Mix A** per well.
Sample well : Add 80µL of **urine sample** per well.Add 160 µL of **Reaction Mix A** per well.
Sample Blank well : Add 80µL of **urine sample** per well. Add 160 µL of **Reaction Mix B** per well.
(2) Mix thoroughly. Incubate for 45minutes at 37°C. Protect from light.
Note : Prepare LDH Solution during this incubation time.
(3) Measure the optical density (S1, A1, B1; refer to figures below) at 340nm of each well using a microplate reader.
Note : Eliminate all bubbles before measuring optical density.
(4) Add 10µL of the **LDH Solution** to each well. Mix thoroughly.
(5) Incubate for 45 minutes at 37°C. Protect from light.
(6) Measure the optical density (S2, A2, B2) at 340nm of each well using a microplate reader. Eliminate all bubbles before measuring optical density.

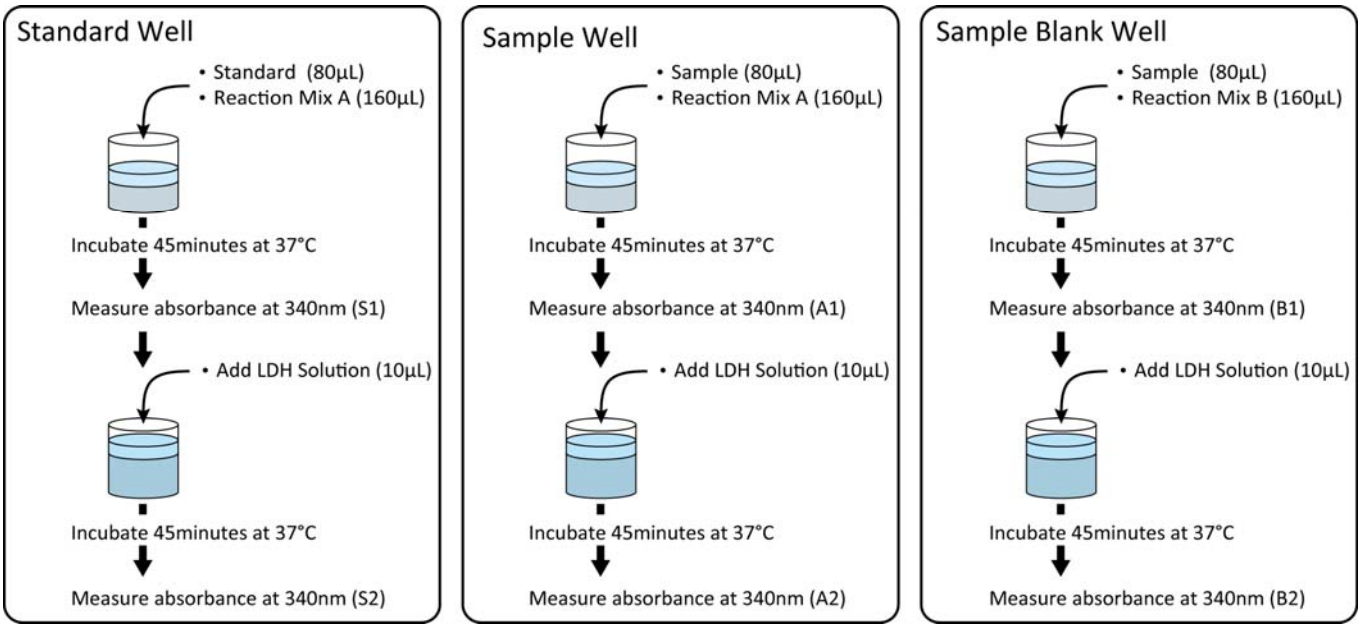

Figure 2 : Overview of the assay procedure.

V. Calculation of results

- Calculate the Net Standard OD by subtracting Standard OD (S2) from Standard OD (S1).
  Net Standard OD = Standard OD (S1) - standard OD (S2)
- Plot the Net Standard OD against the D-serine Standard Concentrations to produce the D-serine standard curve.
- Calculate the Net Sample OD as follows:
  Net Sample OD = [Sample OD (A1) - Sample OD (A2)] - [Sample blank OD (B1) - Sample blank OD (B2)]
- Determine the D-Serine concentration of an unknown sample from its Net Sample OD using the equation for the D-serine standard curve.

< Experimental Example >

An example is shown below. Table 4 shows typical OD results for D-serine standards and the resulting standard curve (Figure 3).

Table 4 : Example assay data with D-serine standards

D-Serine (mM)	OD（S1）	OD（S2）	Net Standard OD (S1-S2)
0	2.040	1.974	0.066
0.05	2.027	1.916	0.111
0.1	2.040	1.867	0.173
0.4	2.049	1.536	0.513
1	2.027	0.857	1.170

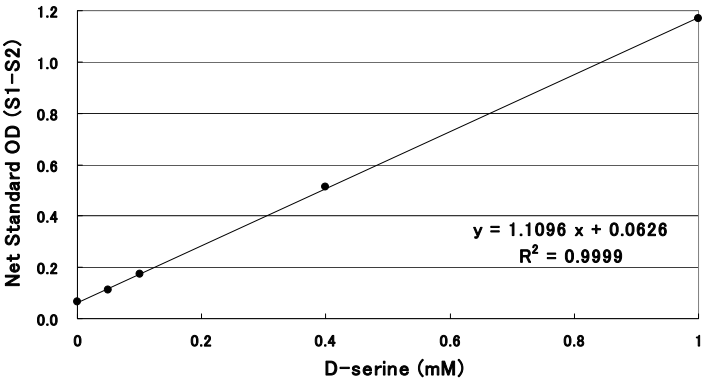

Figure 3 : D-Serine standard curve produced from assay data in Table 4

Table 5 shows example OD results for a urine sample. The net sample OD is 0.281. From the standard curve given in Figure 3, the concentration of D-serine in the sample is 0.197 mM.

Table5 : Example data for an assayed sample.

	OD (A1)	OD (A2)	OD (A1)- OD (A2)	Net Sample OD
Sample	2.602	2.211	0.391	0.281

	OD (B1)	OD (B2)	OD (B1)- OD (B2)
Sample blank	2.678	2.568	0.11

References

(1) Tomokazu Ito, Kei Takahashi, Tomoko Naka, Hisashi Hemmi, Tohru Yoshimura (2007).
Enzymatic assay of D-serine using D-serine dehydratase from Saccharomyces cerevisiae.

(2) Tomokazu Ito, Hisashi Hemmi, Kunishige Kataoka, Yukio Mukai and Tohru Yoshimura (2008).
A novel zinc-dependent D-serine dehydratase from Saccharomyces cerevisiae.

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