Version 2 Last updated 10 August 2023

ab241027
DL-Serine Assay Kit

For the measurement of DL-Serine in plasma, serum, and CSF samples, soft tissue homogenates and cell lysates.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

DL-Serine Assay Kit (ab241027) allows for quantification of both L- and D-Serine in biological fluids and tissues. The assay is based on the conversion of L-Serine to D-Serine, which is metabolized to an intermediate product that is subsequently oxidized and reacts with a probe to form a stable fluorophore (Ex/Em = 535/587 nm). Samples may be divided and assayed simultaneously for quantification of both D-Serine and total DL-Serine. The assay is not affected by physiological concentrations of other amino acids, is high-throughput adaptable and can detect less than 1 µM Serine.

Prepare Samples and Standards as directed.

Prepare D-Serine only and Total Serine Reaction Mixes (samples and standards) and Sample Background Mix (sample controls). Add to samples, standards and controls, as appropriate.

Incubate at 37°C for 60 min, protected from light.

Read fluorescence at Ex/Em = 535/587 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer LII/Serine Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Probe Solution	200 µL	-20°C	-20°C
Serine Racemase Enzyme Mix	1 vial	-20°C	-20°C
D-Serine Enzyme Mix	1 vial	-20°C	-20°C
Serine Developer Mix/Developer Enzyme Mix	1 vial	-20°C	-20°C
Sample Cleanup Mix	1 vial	-20°C	-20°C
D-Serine Standard	1 vial	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multiwell fluorescence microplate reader
- Black 96-well plates with flat bottom
- 10 kDa spin columns.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Assay Buffer LII/Serine Assay Buffer

Ready to use as supplied. Warm to room temperature before use. Store at -20°C.

5.2 OxiRed Probe/Probe Solution

Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature. After use, promptly retighten cap to minimize adsorption of airborne moisture.

Δ Note: *the OxiRed Probe/probe can turn pink if it is exposed to light or absorbs moisture from the atmosphere. Consider wrapping the proble solution in aluminum foil while sitting on the bench.*

5.3 Serine Racemase Enzyme Mix

Reconstitute contents of each vial with 110 µL of Assay Buffer LII/Serine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.

5.4 D-Serine Enzyme Mix

Reconstitute contents of each vial with 110 µL of Assay Buffer LII/Serine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.

5.5 Serine Developer Mix/Developer Enzyme Mix

Reconstitute contents of each vial with 220 µL of Assay Buffer LII/Serine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Keep on ice while in use and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

5.6 Sample Cleanup Mix

Reconstitute contents of each vial with 220 µL of Assay Buffer LII/Serine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Keep on ice while in use and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

5.7 D-Serine Standard

Reconstitute with 110 µL of dH_2O for a 10 mM stock solution. Store at -20°C, stable for 5 freeze/thaw cycles.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 200 µM solution of D-Serine by adding 20 µL of the 10 mM D-Serine Standard stock to 980 µL of Assay Buffer LII/Serine Assay Buffer.
2. Add 0, 2, 4, 6, 8, and 10 µL of the 200 µM working solution into a series of wells, generating 0, 400, 800, 1200, 1600 and 2000 pmol of D-Serine/well.
3. Adjust the volume to 60 µL/well with Assay Buffer LII/Serine Assay Buffer.

Standard #	200 µM D-Serine Standard (µL)	Assay Buffer LII/Serine Assay Buffer (µL)	Final volume standard in well (µL)	D-Serine (pmol/well)
1	0	60	60	0
2	2	58	60	400
3	4	56	60	800
4	6	54	60	1200
5	8	52	60	1600
6	10	50	60	2000

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Preparation:

1. Biological fluid samples (such as plasma and serum) should be clarified by centrifugation at 10,000 x *g* for 5 minutes in order to reduce turbidity and separate insoluble material.
2. Soft tissues (~10 mg) or cultured cells (~1 x 10^6) should be rapidly homogenized on ice with 100 µl ice cold Assay Buffer LII/Serine Assay Buffer. Centrifuge at 15,000 x *g* for 10 minutes at 4°C and transfer the supernatant to a new microfuge tube.
3. Common metabolites found in biological samples may interfere with assay reactions or increase sample background. To eliminate potential sources of interference, samples should be pretreated with Sample Cleanup Mix and deproteinized. For each test sample, add Sample Cleanup Mix to the sample at a 1:25 ratio (4 µL for every 100 µL of sample volume). Incubate samples at 37°C for 15 min, and then transfer samples to 10 kDa MWCO Spin Columns. Centrifuge treated samples at 10,000 x *g* for 10 minutes and collect the filtrate. Once deproteinized, samples may be stored at -20°C for future experiments for at least 2 months.
4. Add 2-20 µL of pretreated, filtered sample to desired well(s) in a black, flat bottom 96-well plate. For each test sample, prepare at least three parallel sample wells: one for determination of D-Serine only, one for determination of total serine (both the D- and L-isomers) and one to serve as a sample background control. Adjust the volume of all wells to 60 µl/well with Assay Buffer LII/Serine Assay Buffer.

ΔNote: Serine concentration can vary dramatically depending upon the sample type. For unknown samples, we recommend performing a pilot experiment to ensure readings are within the standard curve range. Average physiological ranges for total serine

are 60-190 µM in serum, 20-70 µM in CSF and 5-20 µM in saliva. In most mammalian samples, L-Serine accounts for ~95% of the total. **ΔNote:** As physiological concentrations of D-Serine are often very low (between 1-3 µM in plasma/serum and 2-5 µM in CSF), we recommend running two D-Serine Only test samples in parallel and spiking one with a known amount of D-Serine Standard (e.g. 400 pmol) to ensure accurate determination of D-Serine. Addition of a spiked sample brings the number of parallel samples to four.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction mix:

1. Preincubate the plate at 37°C for 10 minutes.
2. During the preincubation period, prepare reaction mixes for D-Serine Only, Total Serine and Sample Background Control wells according to the table below.
3. Prepare a sufficient amount of each type of reaction mix to add 40 µL to all assay wells of that type.

Component	D-Serine Only & Standards Reaction Mix (µL)	Total (D+L)-Serine Reaction Mix (µL)	Sample Background Reaction Mix (µL)
Assay Buffer LII/Serine Assay Buffer	36	35	37
Serine Racemase Enzyme Mix	-	1	-
D-Serine Enzyme Mix	1	1	-
OxiRed Probe/Probe Solution	1	1	1
Serine Developer Mix/Developer Enzyme Mix	2	2	2

ΔNote: Remember to account for the D-Serine Standard wells and any additional wells for spiked samples (if applicable) when calculating the amount of D-Serine Only reaction mix to prepare.

8.2 Measurement:

1. Add 40 µL of the D-Serine Only & Standards Reaction Mix to each well containing the D-Serine Standards and Samples. Add

40 µL of the Total (D+L)-Serine Reaction Mix to each well containing duplicate Samples.

2. Add 40 µL of the sample background mix into Sample Background Control wells.
3. Incubate the plate at 37°C for 60 minutes, protected from light.
4. Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 535/587 nm in endpoint mode.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each Standard, Control and Sample.
2. Correct background by subtracting the value of the 0 D-Serine Standard from all Sample readings.
3. If significant, subtract the Sample Background Control from sample readings.
4. Plot the standard curve.
5. Apply the corrected sample readings to the standard curve to get the amount of D-Serine in the sample wells.
6. Concentration of D-Serine (pmol) in the test samples is calculated as:

$$\text{D - Serine or Total DL - Serine concentration(C)} = \frac{B}{V} * D$$

Where:

C = Serine concentration in test sample (pmol/µl = µM).

B = amount of Serine in the sample from the Standard Curve (pmol).

V= sample volume added to the sample well (µL).

D = dilution factor

ΔNote: To quantify sample L-Serine level, subtract the amount of D-Serine detected from the total DL-Serine amount and calculate the concentration based upon the sample volume and dilution factor (if applicable) as above.

For spiked D-Serine Only samples, calculate B by subtracting the background corrected non-spiked sample reading (Fs) from the corrected spiked reading (Fs+spike):

$$Amount\ of\ D-Serine\ in\ spiked\ sample\ wells\ (B)$$

$$= \left(\frac{Fs}{(Fs + spike) - Fs}\right) \times\ D-Serine\ Spike\ (in\ pmol)$$

10. Typical Data

Data provided **for demonstration purposes only.**

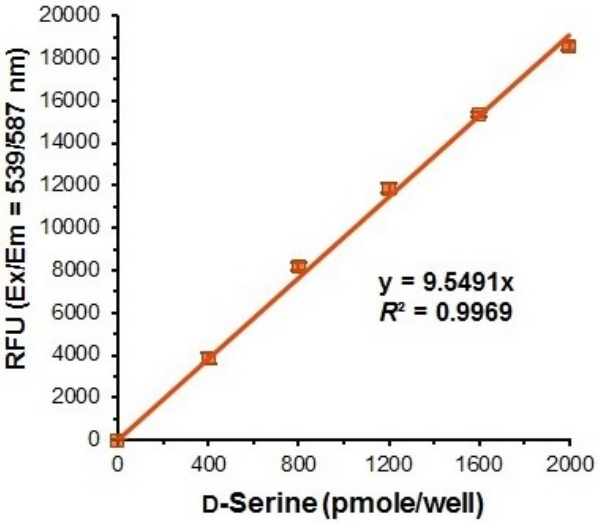

Figure 1. D-Serine Standard curve

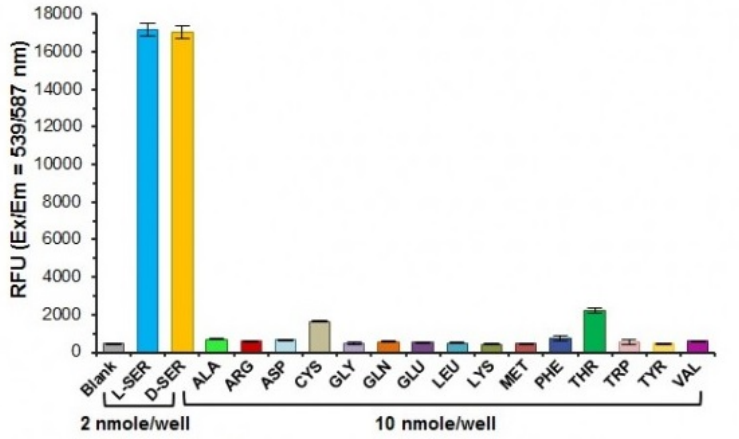

Figure 2. Specificity for detection of L- or D-Serine (SER) over other amino acids. At a 5-fold molar excess (10 nmoles) versus SER isomers (each 2 nmoles), L-threonine (THR) contributes ≤15% interference, L-cysteine (CYS) contributes ≤10% interference and all other amino acids contribute ≤5%.

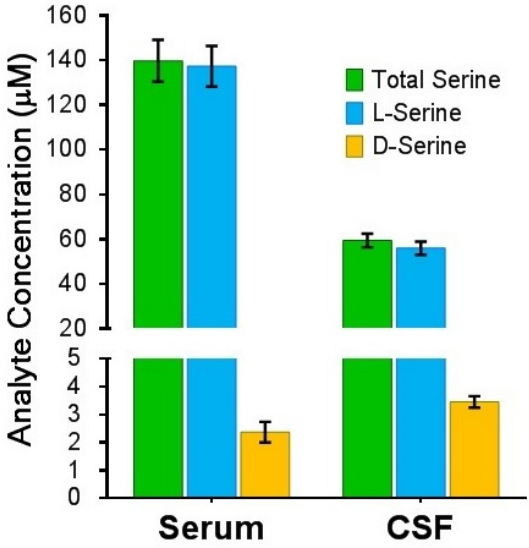

Figure 3. Estimation of total, D- and L-Serine in pooled normal human serum and CSF (10 µL). L-Serine concentrations for serum and CSF samples were 137.4 ± 9.06 µM and 56.01 ± 2.93 µM, whereas D-Serine concentrations were 2.37 ± 0.36 µM and 3.46 ± 0.21 µM, respectively. Data are mean ± SEM of 3 replicates, assayed according to the kit protocol.

11. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)