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Product Information

DL-Serine Assay Kit

Catalog Number **MAK352**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Serine is one of the 20 naturally-occurring amino acids used by all organisms in the biosynthesis of proteins. Having a single chiral center, serine can exist as one of two stereoisomers (L-Serine and D-Serine). In addition to its role as a protein building block, L-Serine serves as a precursor to many vital biomolecules, including phosphatidylserine, sphingomyelin, and the amino acids glycine and cysteine. The non-proteogenic isomer D-Serine is also synthesized from L-Serine in the mammalian brain. While most D-amino acids are only present in trace amounts in mammals, D-Serine is a vital neurotransmitter that acts as a co-agonist of synaptic *N*-methyl-D-aspartate (NMDA) type glutamate-gated ion channels. Impaired D-Serine metabolism may contribute to several neuropsychiatric disorders, including depression, schizophrenia, epilepsy, and dementia.

The DL-Serine Assay Kit 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 (λ_{ex} = 535 nm/ λ_{em} = 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.

The kit is suitable for the estimation of D- and L-Serine concentrations in human or animal biological fluids (plasma, serum, CSF, etc.), soft tissue homogenates (i.e., liver, brain, etc.) and cultured cell lysates (adherent or suspension cells).

Components

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

Serine Assay Buffer Catalog Number MAK352A	25 mL
Probe Solution Catalog Number MAK352B	200 μL
Serine Racemase Enzyme Mix Catalog Number MAK352C	1 vial
D-Serine Enzyme Mix Catalog Number MAK352D	1 vial
Developer Enzyme Mix Catalog Number MAK352E	1 vial
Sample Cleanup Mix Catalog Number MAK352F	1 vial
D-Serine Standard Catalog Number MAK352G	1 vial

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flatbottom 96 well plates
- Fluorescence multiwell plate reader, capable of 37 °C temperature setting
- Refrigerated microcentrifuge capable of RCF ≥15,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

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, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.
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.

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

Developer Enzyme Mix and Sample Cleanup Mix – Reconstitute contents of each vial with 220 µL of 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.

D-Serine Standard – Reconstitute with 110 µL of ultrapure water for a 10 mM stock solution. Store at –20 °C, stable for 5 freeze/thaw cycles.

Procedure
Sample Preparation
Note: Serine concentration can vary dramatically depending upon the sample type. For unknown samples, perform 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.

Biological fluid samples (such as plasma and serum)
Clarify by centrifugation at 10,000 × *g* for 5 minutes in order to reduce turbidity and separate insoluble material.

Soft tissues (~10 mg) or cultured cells (~1 x 10^6)
Rapidly homogenize on ice with 100 µL of ice cold Serine Assay Buffer. Centrifuge at 15,000 × *g* for 10 minutes at 4 °C and transfer the supernatant to a new microfuge tube.

Sample Cleanup
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 minutes, and then transfer samples to a Corning Spin-X UF concentrator. Centrifuge treated samples at 10,000 × *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.

Sample Addition to Wells
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 Serine Assay Buffer.

Note: As physiological concentrations of D-Serine are often very low (between 1–3 µM in plasma and serum, and 2–5 µM in CSF), it is recommended to run 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.

Standard Curve Preparation
Prepare a 200 µM solution of D-Serine by adding 20 µL of the 10 mM D-Serine Standard to 980 µL of Serine Assay Buffer. Prepare D-Serine Standards according to Table 1. Mix well.

Table 1.
Preparation of D-Serine Standards

Well	200 µM Premix	Serine Assay Buffer	D-Serine (pmol/well)
1	0 µL	60 µL	0
2	2 µL	58 µL	400
3	4 µL	56 µL	800
4	6 µL	54 µL	1,200
5	8 µL	52 µL	1,600
6	10 µL	50 µL	2,000

Reaction Mix

1. Preincubate the plate at 37 °C for 10 minutes, protected from light.
2. During the preincubation period, prepare reaction mixes for D-Serine Only, Total Serine, and Sample Background Control wells according to Table 2, mix well. Make a sufficient amount of each type of reaction mix to add 40 µL to all assay wells of that type. 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.
3. Add 40 µL of D-Serine Only reaction mix to standard curve and D-Serine Only test sample wells.
4. Add 40 µL of Total Serine reaction mix to corresponding test sample wells.
5. For sample background control wells, add 40 µL of the Sample Background reaction mix.

Table 2.
Preparation of Reaction Mixes

Reagent	D-Serine Only and Standards	Total Serine (D+L)	Sample Background
Serine Assay Buffer	36 µL	35 µL	37 µL
Serine Racemase Enzyme Mix	–	1 µL	–
D-Serine Enzyme Mix	1 µL	1 µL	–
Probe Solution	1 µL	1 µL	1 µL
Developer Enzyme Mix	2 µL	2 µL	2 µL

Measurement

Incubate the plate at 37 °C for 60 minutes, **protected from light**. Measure the fluorescence of all sample, background and standard curve wells at λ_{ex} = 535 nm/λ_{em} = 587 nm in endpoint mode.

Results

1. For the D-Serine Standard curve, subtract the zero standard (0 pmol/well) reading from all of the standard readings, plot the background-subtracted values, and calculate the slope of the standard curve.
2. For test samples, calculate the corrected sample fluorescence (F_s) by subtracting the Sample Background RFU reading from the corresponding D-Serine Only and Total Serine sample readings: $F_s = RFU_S - RFU_{BC}$.
3. For unspiked samples, apply the F_s values to the standard curve to get B pmol of Serine (either D-Serine or total DL-Serine, depending upon the sample reaction type) in the well.

Sample D-Serine or Total DL-Serine Concentration (pmol/µL or µM) =

$$(B/V) \times D$$

where:
B = the amount of serine, calculated from the standard curve (in pmol)
V = volume of sample added to the well (in µL)
D = sample dilution factor (if applicable, D = 1 for undiluted samples)

4. 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 (F_s) from the corrected spiked reading ($F_{s+spike}$).

Amount of D-Serine in spiked sample wells

$$(B) = \left(\frac{F_s}{(F_{s+spike}) - F_s}\right) \times \textit{D-Serine Spike} \text{ (in pmol)}$$

Figure 1.
Typical D-Serine Standard curve

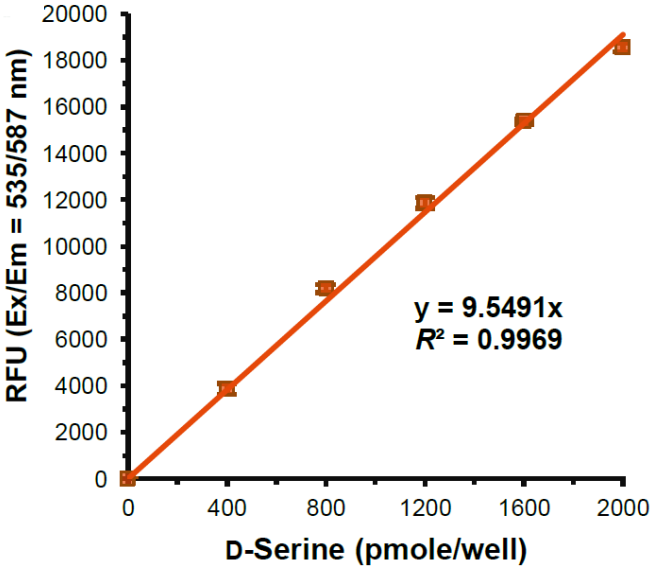
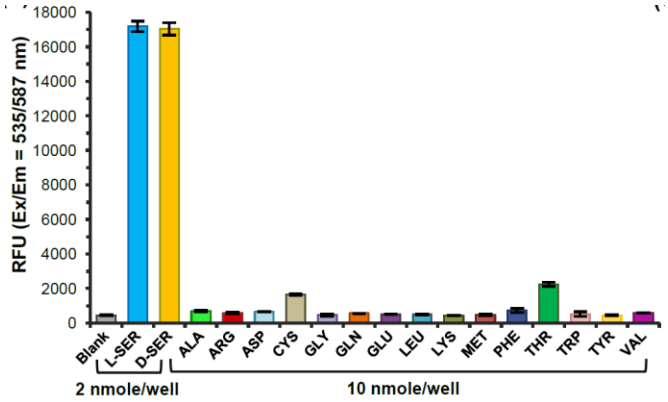
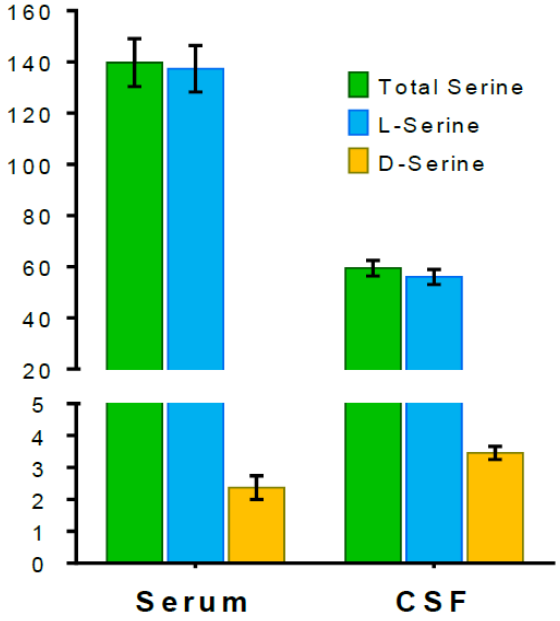


Figure 2.
Specificity for Detection of L- or D-Serine



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



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 procedure.

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