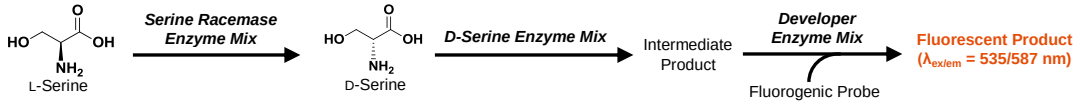
DL-Serine Assay Kit (Fluorometric)

(Catalog # K743-100; 100 Reactions; Store at -20°C)

I. Introduction:

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 either 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. BioVision's 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 (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.



II. Applications:

- Estimation of D- and L-Serine concentration in various biological samples

III. Sample Type:

- Human or animal biological fluids (plasma, serum, CSF, etc.)
- Soft tissue homogenates (*i.e*. liver, brain, etc.)
- Cultured cell lysates (adherent or suspension cells)

IV. Kit Contents:

Components	K743-100	Cap Code	Part Number
Serine Assay Buffer	25 ml	WM	K743-100-1
Probe Solution	200 µl	Red	K743-100-2
Serine Racemase Enzyme Mix	1 vial	Blue	K743-100-3
D-Serine Enzyme Mix	1 vial	Green	K743-100-4
Developer Enzyme Mix	1 vial	Amber	K743-100-5
Sample Cleanup Mix	1 vial	Clear	K743-100-6
D-Serine Standard	1 vial	Yellow	K743-100-7

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- Black 96-well plates with flat bottom
- 10 kDa Spin Column (Cat. # 1997 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Serine Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

- **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 dH_2O for a 10 mM stock solution. Store at -20°C, stable for 5 freeze/thaw cycles.

VII. Serine Assay Protocol:

1. Sample Preparation:

a. Biological fluid samples (such as plasma and serum) should be clarified by centrifugation at 10,000 x *g* for 5 min in order to reduce turbidity and separate insoluble material. Soft tissues (~10 mg) or cultured cells (~1 x 10^6) should be rapidly homogenized on ice with 100 µl ice cold Serine Assay Buffer. Centrifuge at 15,000 x *g* for 10 min at 4°C and transfer the supernatant to a new microfuge tube.

b. 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 (Cat. # 1997 or equivalent). Centrifuge treated samples at 10,000 x *g* for 10 min and collect the filtrate. *Once deproteinized, samples may be stored at -20°C for future experiments for at least 2 months.*

c. 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.

Notes:

- 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.
- 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.*

2. **Standard Curve Preparation:** Prepare a 200 µM solution of D-Serine by adding 20 µl of the 10 mM D-Serine Standard stock to 980 µl of Serine Assay Buffer. 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. Adjust the volume to 60 µl/well with Serine Assay Buffer.
3. **Reaction Mix Preparation:**
   a. Preincubate the plate at 37°C for 10 min, protected from light. During the preincubation period, prepare reaction mixes for D-Serine Only, Total Serine and Sample Background Control wells according to the table below. 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.*

	D-Serine Only & Standards	Total (D+L)-Serine	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

   b. Add 40 µl of D-Serine Only reaction mix to standard curve and D-Serine Only test sample wells. Add 40 µl of Total Serine reaction mix to corresponding test sample wells. For sample background control wells, add 40 µl of the Sample Background reaction mix.
4. **Measurement:** Incubate the plate at 37°C for 60 min, **protected from light**. Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 535/587 nm in endpoint mode.
5. **Calculations:** 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. 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: $F_S = RFU_S - RFU_{BC}$. 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.

$$\textbf{Sample D-Serine or Total DL-Serine Concentration} = \frac{B}{V} \times D = \textbf{pmol/µl = µM}$$

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

Note: 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 ($F_{S+spike}$):

$$\text{Amount of D-Serine in spiked sample wells } (B) = \left(\frac{F_S}{(F_{S+spike}) - F_S}\right) \times \textit{D-Serine Spike} \text{ (in pmol)}$$

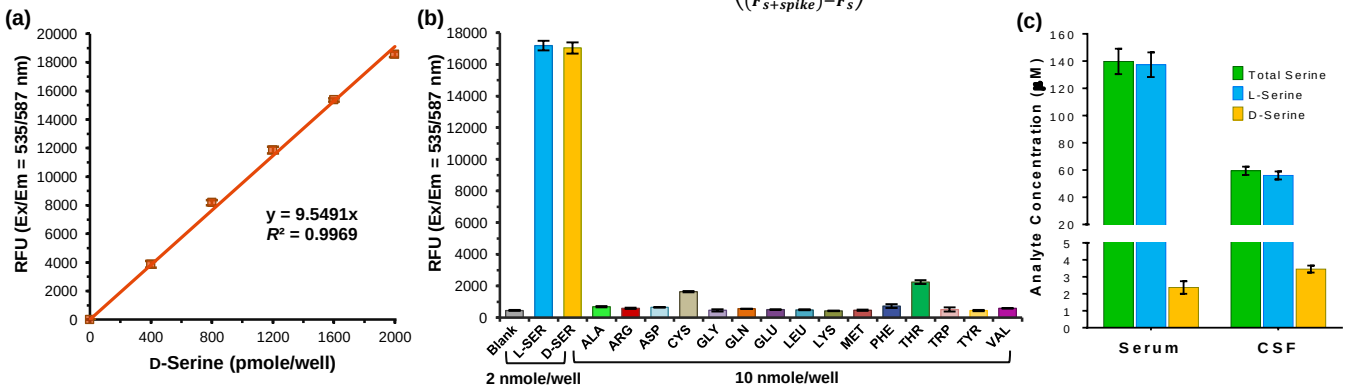


Figure: (a) D-Serine Standard curve. **(b)** Specificity for detection of L- or D-Serine (SER) over other amino acids. At a 5-fold molar excess (10 nmoles) versus SER isomers (each 2 nmoles), L-threonine (THR) contributes ≤15% interference, L-cysteine (CYS) contributes ≤10% interference and all other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

VIII. RELATED PRODUCTS:

Glycine Assay Kit (K589)	Glutamine Assay Kit (K556)	Cysteine Assay Kit (K558)	Glutamate Assay Kit (K629)
Alanine Assay Kit (K652)	Tyrosine Assay Kit (K573)	Aspartate Assay Kit (552)	Phenylalanine Assay Kit (K572)

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