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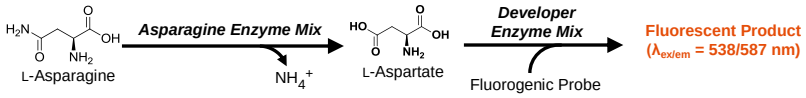
Asparagine Assay Kit (Fluorometric)

Rev 09/19

(Catalog # K736-100; 100 Assays; Store at -20°C)

I. Introduction:

Asparagine is one of the 20 naturally-occuring amino acids used by all organisms in the biosynthesis of proteins. In humans, it is considered a non-essential amino acid, as it can be synthesized *in vivo* from aspartate and glutamine. In addition to its role as a protein building block, asparagine is vital for pre- and postnatal neuronal development. Congenital deficiency of the enzyme Asparagine Synthetase (EC 6.3.5.4) results in microcephaly, intractable seizures and severe neurodevelopmental disabilities. In contrast, depletion of asparagine levels is employed as a treatment strategy for certain cancers, particularly acute lymphoblastic leukemia (ALL) and other hematopoietic cancers. Lymphoblasts are incapable of synthesizing asparagine *de novo* and are thus dependent on exogenous sources. Treatment with the bacterial enzyme Asparaginase (EC 3.5.1.1) deprives malignant cells of the circulating plasma asparagine needed for rapid growth. BioVision's Asparagine Assay Kit allows for highly sensitive quantification of L-asparagine levels in biological fluids and cultured cells. In the assay, L-asparagine is hydrolysed to generate aspartate, which is converted to pyruvate and subsequently reacts with a fluorogenic probe to form a stable fluorophore (Ex/Em = 538/587 nm) via a series of coupled enzymatic reactions. The assay is not affected by physiological concentrations of other common amino acids and can detect asparagine levels down to 5 µM in samples.



II. Applications:

- Estimation of L-Asparagine concentration in various biological samples

III. Sample Type:

- Human or animal biological fluids (plasma, serum, CSF, etc.)
- Cultured cell lysates (adherent or suspension cells) or cell culture growth medium

IV. Kit Contents:

Components	K736-100	Cap Code	Part Number
Asparagine Assay Buffer	25 ml	WM	K736-100-1
Probe Solution	200 µl	Red	K736-100-2
Asparagine Enzyme Mix	1 vial	Blue	K736-100-3
Conversion Enzyme Mix	1 vial	Green	K736-100-4
Developer Enzyme Mix	1 vial	Violet	K736-100-5
Sample Cleanup Mix	1 vial	Clear	K736-100-6
L-Asparagine Standard	1 vial	Yellow	K736-100-7

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 Asparagine 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.
- **Asparagine Enzyme Mix:** Reconstitute with 220 µl of Asparagine Assay Buffer. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.
- **Conversion Enzyme Mix**, **Developer Enzyme Mix** and **Sample Cleanup Mix:** Reconstitute each vial with 220 µl of Asparagine 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.
- **L-Asparagine Standard:** Reconstitute with 110 µl of ddH_2O for a 10 mM stock solution. Store at -20°C, stable for 4 freeze/thaw cycles.

VII. Asparagine Assay Protocol:

1. Sample Preparation:

a. Biological fluid samples (such as plasma and serum) or cell culture medium should be clarified by centrifugation at 10,000 x *g* for 5 min at 4°C in order to separate insoluble material. Cultured cells (~1 x 10^6) should be rapidly homogenized on ice with 100 µl ice cold Asparagine 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 small molecule metabolites (such as pyruvate) and enzymes found in biological samples may interfere with the assay or increase sample background. To reduce potential 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). Mix well and incubate samples at 37°C for 20 min, then transfer samples to 10 kDa MWCO Spin Columns (Cat. # 1997 or equivalent). Centrifuge treated samples at 10,000 x *g* for 10 min at 4°C and collect the filtrate. *Once pretreated and deproteinized, samples may be stored at -80°C for future experiments for at least 2 months.*

c. Add 2-30 µl of sample to desired well(s) in a black, flat bottom 96-well plate. For each test sample, we recommend preparing *two parallel sample wells*, with one well serving as a sample background control. Adjust the volume of all wells to 60 µl/well with Asparagine Assay Buffer.

Notes:

- Asparagine 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 L-Asparagine are 15-60 µM in blood serum/plasma and 5-15 µM in CSF.
- For samples with a low Asparagine concentration, we recommend running two test samples in parallel and spiking one with a known amount of L-Asparagine Standard (*e.g.* 400 pmol) to ensure accurate determination of L-Asparagine. *Addition of a spiked sample brings the number of parallel sample wells to three.*

2. **Standard Curve Preparation:** Prepare a 200 µM solution of L-Asparagine by adding 20 µl of the 10 mM L-Asparagine Standard stock to 980 µl of Asparagine 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 L-Asparagine/well. Adjust the volume to 60 µl/well with Asparagine Assay Buffer.
3. **Reaction Mix Preparation:**
   a. Prepare reaction mixes for Sample and 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 L-Asparagine Standard curve wells and any additional wells for spiked samples (if applicable) when calculating the amount of reaction mix to prepare.*

	Samples & Standards	Sample Background
Asparagine Assay Buffer	33 µl	35 µl
Asparagine Enzyme Mix	2 µl	—
Conversion Enzyme Mix	2 µl	2 µl
Developer Enzyme Mix	2 µl	2 µl
Probe Solution	1 µl	1 µl

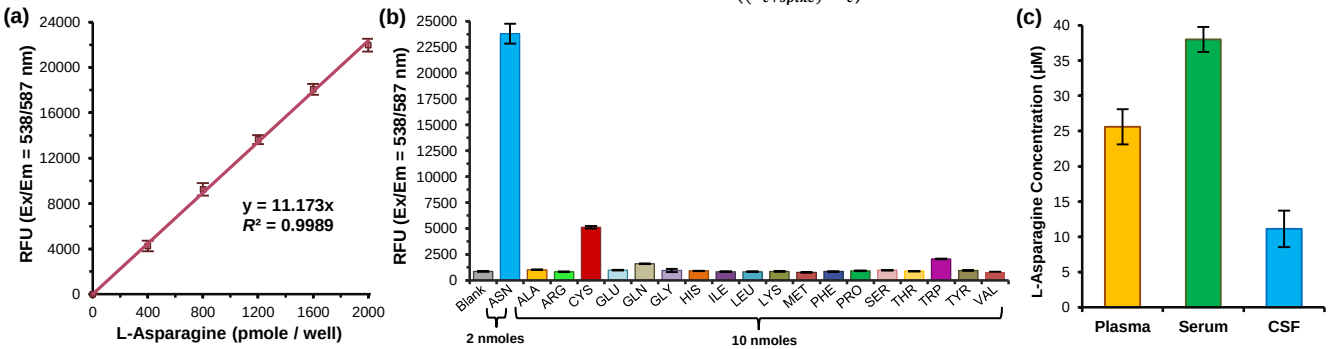
   b. Add 40 µl of reaction mix to all standard curve and test sample wells. For sample background control wells, add 40 µl of the Sample Background reaction mix.
4. **Measurement:** Incubate the plate for 25 min at room temperature, **protected from light**. Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 538/587 nm in endpoint mode.
5. **Calculations:** For the L-Asparagine 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_c) by subtracting the Sample Background RFU reading from the corresponding sample readings: $F_c = RFU_S - RFU_{BC}$. For unspiked samples, apply the F_c values to the standard curve to get *B* pmol of Asparagine in the well.

$$\textbf{Sample L-Asparagine Concentration} = \frac{B}{V} \times D = \textbf{pmol/µl = µM}$$

Where: ***B*** is the amount of Asparagine, 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 Asparagine level in spiked samples (if applicable), calculate *B* by subtracting the background corrected non-spiked sample reading (F_c) from the corrected spiked reading ($F_{c+spike}$):

$$\text{Amount of L-Asparagine in spiked sample wells } (\boldsymbol{B}) = \left(\frac{F_c}{(F_{c+spike}) - F_c}\right) \times \textit{Asparagine Spike} \text{ (in pmol)}$$



Figures: (a) L-Asparagine Standard curve. **(b)** Specificity for detection of L-asparagine (ASN) over other common L-amino acids. At a 5-fold molar excess (10 nmole/well) versus ASN (2 nmole/well), tryptophan (TRP) gives ~5% interference and the reduced form of cysteine (CYS) gives ~20% interference (the oxidized disulfide form, which accounts for 90% of the total cysteine in biological fluids, generates ≤1% interference at 5-fold excess). **(c)** Estimation of total asparagine in pooled heparinized human plasma (10 µl), pooled off-the-clot human serum (10 µl) and pooled human CSF (15 µl). Asparagine concentrations for plasma, serum and CSF samples were 25.61 ± 2.51 µM, 37.99 ± 1.77 µM and 11.11 ± 2.59 µM, respectively. Data are mean ± SEM of at least 3 replicates, samples were treated with Sample Cleanup Mix, deproteinized using 10 kDa MWCO spin columns (Cat. # 1997) and 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 (K552)	Phenylalanine Assay Kit (K572)
DL-Serine Assay Kit (K743)	Homocysteine Assay Kit (K531)	Methionine Assay Kit (K442)	Tryptophan Assay Kit (K557)
Threonine Assay Kit (K463)	BCAA Assay Kit (K564)	Taurine Assay Kit (K988)	L-Arginine Assay Kit (K749)

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