Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

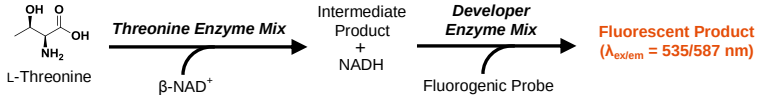
PicoProbe™ Threonine Assay Kit (Fluorometric)

rev 11/19

(Catalog # K463-100; 100 Assays; Store at -20°C)

I. Introduction:

Threonine is one of the 20 naturally-occuring amino acids used by all organisms in the biosynthesis of proteins and is an essential dietary amino acid for humans. Threonine residues in proteins are subjected to post-translational phosphorylation, a critical regulatory and signal transduction mechanism. In addition to its role as a protein building block, threonine can serve as a precursor to other amino acids and metabolites, including glycine, isoleucine and acetyl-coenzyme A. Recent research has shown that threonine is a vital nutrient for embryonic stem cells and threonine metabolism (mediated by the enzyme threonine dehydrogenase) and is required for unlimited self-renewal and maintenance of stem cell pluripotency. Dietary threonine deficiency, while rare, can result in breakdown of the intestinal mucosal lining (eventually triggering inflammatory bowel disorders), immunosuppression and a multitude of neuropsychiatric symptoms. BioVision's Threonine Assay Kit allows for highly sensitive quantification of L-Threonine levels in biological fluids and tissues. The assay is based on the selective, NAD^+-coupled enzymatic metabolism of threonine, yielding an oxidized intermediate and NADH. A developer enzyme mixture utilizes the NADH generated to convert PicoProbe™ into a stable fluorophore (Ex/Em = 535/587 nm). The assay is not affected by physiological concentrations of other amino acids, is high-throughput adaptable and can detect threonine levels down to 2 µM in samples.



II. Applications:

- Estimation of L-Threonine 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) or cell culture growth medium

IV. Kit Contents:

Components	K463-100	Cap Code	Part Number
Threonine Assay Buffer	25 ml	WM	K463-100-1
PicoProbe™ Solution	200 µl	Blue	K463-100-2
Threonine Enzyme Mix	1 vial	Green	K463-100-3
Developer Enzyme Mix	1 vial	Red	K463-100-4
L-Threonine Standard	1 vial	Yellow	K463-100-5

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- White 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 Threonine Assay Buffer to warm to room temperature (RT) prior to use. Read the entire protocol before performing the assay procedure.

- **PicoProbe™ Solution:** Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to RT. After use, promptly retighten cap to minimize adsorption of airborne moisture.
- **Threonine Enzyme Mix:** Reconstitute with 220 µl of Threonine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.
- **Developer Enzyme Mix:** Reconstitute with 220 µl of Threonine 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-Threonine Standard:** Reconstitute with 110 µl of ddH_2O for a 10 mM stock solution. Store at -20°C, stable for 5 freeze/thaw cycles.

VII. Threonine 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 at 4°C in order to 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 Threonine Assay Buffer. Centrifuge at 15,000 x *g* for 10 min at 4°C and transfer the supernatant to a new microfuge tube.

b. Various enzymes found in biological samples may interfere with the assay. To eliminate potential enzymatic interference, samples should be deproteinized using 10 kDa MWCO Spin Columns (Cat. # 1997 or equivalent). Transfer clarified samples to Spin Columns, centrifuge at 10,000 x *g* for 10 min at 4°C and collect the filtrate. *Once deproteinized, samples may be stored at -20°C for future experiments for at least 2 months.*

c. Add 2-30 µl of sample to desired well(s) in a white, 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 Threonine Assay Buffer.

Notes:

- Threonine 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-threonine are 60-240 µM in serum/plasma, 25-75 µM in CSF and 3-30 µM in saliva.

- For samples with a very low threonine concentration, we recommend running two test samples in parallel and spiking one with a known amount of L-Threonine Standard (*e.g.* 200 pmol) to ensure accurate determination of L-Threonine. *Addition of a spiked sample brings the number of parallel sample wells to three.*

2. **Standard Curve Preparation:** Prepare a 100 µM solution of L-Threonine by adding 10 µl of the 10 mM L-Threonine Standard stock to 990 µl of Threonine Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of the 100 µM working solution into a series of wells, generating 0, 200, 400, 600, 800 and 1000 pmol of L-Threonine/well. Adjust the volume to 60 µl/well with Threonine Assay Buffer.
3. **Reaction Mix Preparation:**
   a. Preincubate the plate at 37°C for 10 min, protected from light. During the pre-incubation period, 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-Threonine 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
Threonine Assay Buffer	35 µl	37 µl
Threonine Enzyme Mix	2 µl	—
PicoProbe™ Solution	1 µl	1 µl
Developer Enzyme Mix	2 µl	2 µl

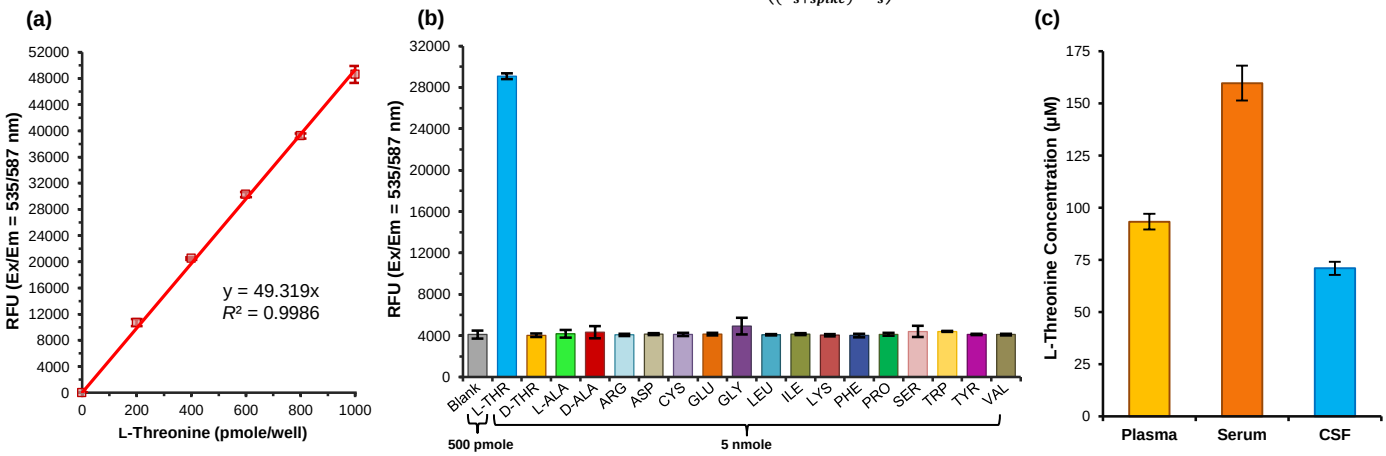
   b. Add 40 µl of reaction mix to all standard curve and test sample wells. For sample background control wells, add 40 µl of the Sample Background reaction mix.
4. **Measurement:** Incubate the plate at 37°C for 60 min, **protected from light**. Measure the fluorescence of all sample, background and Standard Curve wells at Ex/Em = 535/587 nm in endpoint mode.
5. **Calculations:** For the L-Threonine 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 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 Threonine in the well.

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

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

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



Figures: (a) L-Threonine Standard curve. **(b)** Specificity for detection of L-Threonine (L-THR) over D-Threonine and other common amino acids. At a 10-fold molar excess (5 nmole/well) versus L-Threonine (500 pmole/well), all other amino acids tested contribute ≤5% interference. **(c)** Estimation of total L-Threonine in pooled normal human plasma (10 µl), single donor off-the-clot human serum (5 µl) and pooled human CSF (10 µl). L-Threonine concentrations for plasma, serum and CSF samples were 93.26 ± 3.72 µM, 159.7 ± 8.36 µM and 70.92 ± 3.12 µM, respectively. Data are mean ± SEM of at least 3 replicates, samples were 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)
BCAA Assay Kit (K564)	Taurine Assay Kit (K988)		

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