Version 3a, Last updated 10 June 2025

ab239726
Threonine Assay Kit

For the Estimation of L-Threonine concentration in various biological samples.

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

Table of Contents

1. Overview

The Threonine Assay Kit (ab239726) 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 solution/developer enzyme mixture utilizes the NADH generated to convert the probe 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.

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

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Deproteinize all samples and store at -20°C

Add 2-30 µL of sample to desired wells, adjust volume to 60 µL with Threonine Assay Buffer.

Add 40 µL of reaction mix to all assay wells containing samples, standards and controls.

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

Examine cells using light and fluorescence microscope (Ex/Em = 535/587 nm) in endpoint mode.

3. 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.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Threonine Assay Buffer	25 mL	-20°C
PicoProbe II	0.2 mL	-20°C
Threonine Enzyme Mix	1 vial	-20°C
Developer Solution X	1 vial	-20°C
L-Threonine Standard	1 vial	-20°C

PLEASE NOTE: PicoProbe II was previously labelled as Probe solution, and Developer Solution X as Developer Enzyme Mix. The composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well plate with flat bottom.
- Multi-well spectrophotometer
- 10 kDa Spin Column

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Threonine Assay Buffer:

Ready to use as supplied. Bring to room temperature (RT) before use.

6.2 PicoProbe II:

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.

6.3 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.

6.4 Developer Solution X:

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.

6.5 L-Threonine Standard:

Reconstitute with 110 µl of ddH2O for a 10 mM stock solution. Store at -20°C, stable for 5 freeze/thaw cycles.

7. Standard Preparation

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

7.1 Prepare a 100 µM solution of L-Threonine by adding 10 µl of the 10 mM L-Threonine Standard stock to 990 µl of Serine Assay Buffer.

7.2 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.

Standard #	100 µM L-Threonine Standard (µL)	ddH_20 (µL)	Bile Acids/well
1	10	50	1000 pmol
2	8	52	800 pmol
3	6	54	600 pmol
4	4	56	400 pmol
5	2	58	200 pmol
6	0	60	0 pmol

8. Sample Preparation

- For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the 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.

8.1 Biological Fluid Samples

- Fluids 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 106) 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.

8.2 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.

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.

8.3 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.

9. Assay Procedure

9.1 Preincubate the plate at 37°C for 10 min, protected from light.

9.2 During the preincubation 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.

ΔNote: 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 and standards	Sample Background
Threonine Assay Buffer	35 µL	37 µL
Threonine Enzyme Mix	2 µL	-
PicoProbe II	1 µL	1 µL
Developer Solution X	2 µL	2 µL

9.3 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.

9.4 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.

10. Data Analysis

10.1 Subtract the zero L-Threonine Standard curve from all Standard readings. Plot the Standard Curve.

10.2 test samples, calculate the corrected sample fluorescence (Fs) by subtracting the Sample Background RFU reading from the corresponding sample readings: $F_s = RFU_S – RFU_{BC}$.

10.3 For unspiked samples, apply the F_s values to the standard curve to get B pmol of Threonine in the well.

Sample L-Threonine Concentration (C) = B/V X D = pmol/µL or µM

Where: **B** is the amount of threonine, in the sample well from Standard Curve (pmol)
V is sample volume added into the reaction well (µL)

D is sample dilution factor

Δ Note: For spiked samples, correct for any sample interference by using the following equation: Where F_s is the background corrected non-spiked sample reading and $F_{s+spike}$ is the corrected spiked reading.

TBA amount in spiked sample wells (B) =

$$\frac{F_s}{(F_{s+spike})-(F_s)} \times \text{Threonine spike (pmol)}$$

11. Typical Data

Typical data provided **for demonstration purposes only**.

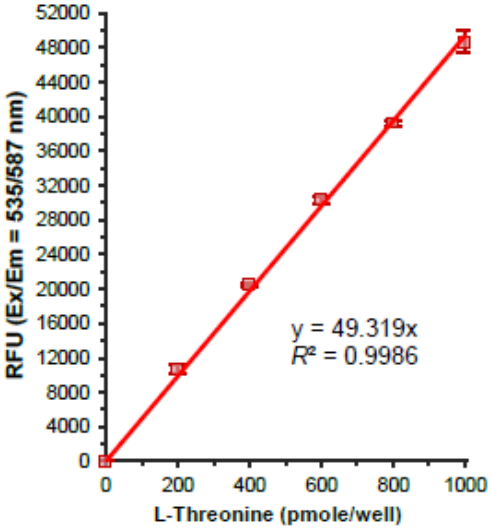

Figure 1. Threonine Standard Curve.

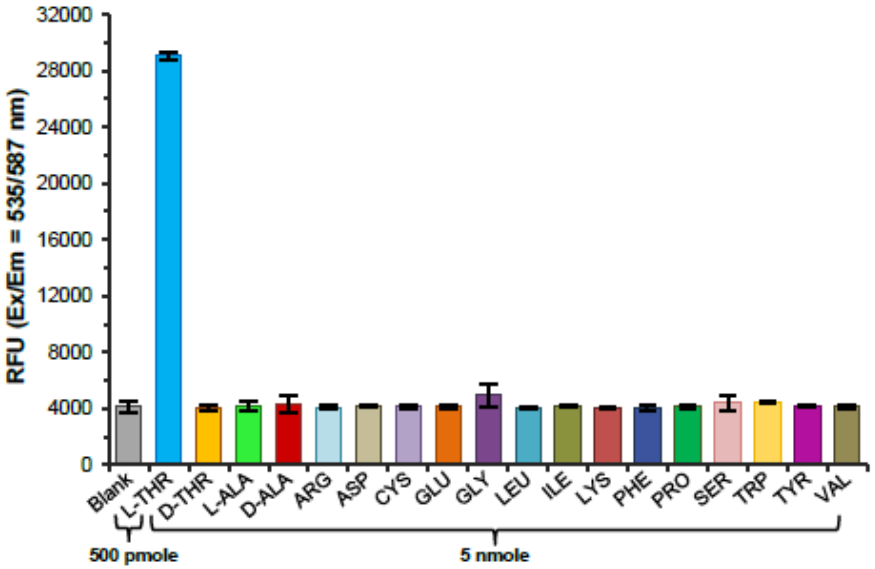

Figure 2. Specificity for detection of L-Threonine (L-THR) over D-Threonine and other common amino acids. At a 10-fold molar excess (5 nmole/well) versus L-Threonine (500 pmole/well), all other amino acids tested contribute ≤5% interference.

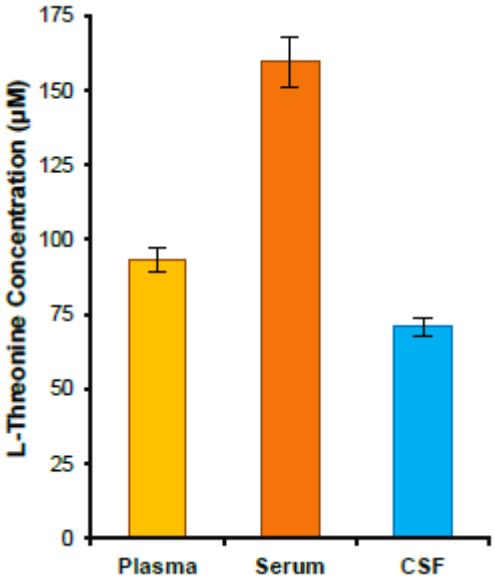

Figure 3. Estimation of total L-Threonine in pooled normal human plasma (10 µl), single donor off-the-clot human serum (5 µl) and pooled human CSF (10 µl). L-Threonine concentrations for plasma, serum and CSF samples were 93.26 ± 3.72 µM, 159.7 ± 8.36 µM and 70.92 ± 3.12 µM, respectively. Data are mean ± SEM of at least 3 replicates, samples were deproteinized using 10 kDa MWCO spin columns and assayed according to the kit protocol.

13. 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)