Product Manual

Threonine Assay Kit

Catalog Number

MET-5205 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Threonine, an essential amino acid, contains a side-chain hydroxyl group, making it polar. It plays a role in the metabolism of fats, the creation of proteins, the proliferation/differentiation of embryonic stem cells, and the function of the intestines. Threonine can be phosphorylated by threonine kinase, and in bacteria it is O-phosphorylated by a kinase, leading to the biosynthesis of cobalamin. It is converted to glycine during the synthesis of L-carnitine in the brain and liver of rats.

Cell Biolabs' Threonine Assay Kit is a simple assay for measuring threonine levels in biological samples. Threonine levels may be quantified in a wide range of biological samples including serum and plasma. The kit has a detection sensitivity limit of 3.1 µM threonine. Each kit provides sufficient reagents to perform up to 100 assays*, including standard curve and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with threonine dehydrogenase (+ThrDH) and one without (-ThrDH). The threonine level is calculated from the difference in OD readings at a wavelength of 450 nm from these 2 assays.

Assay Principle

In the Threonine Assay Kit, ThrDH enzyme converts threonine and NAD+ to L-2-amino-3-ketobutyrate and NADH (Figure 1). The Colorimetric Probe reacts with the reduced NADH, and the absorbance of the plate is read at 450 nm. The content of threonine in the unknown samples is determined by comparison with a predetermined Threonine standard curve.

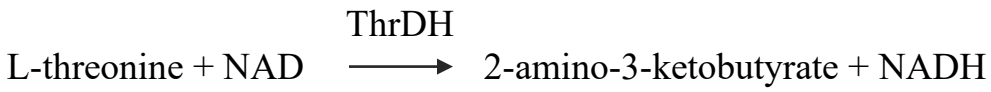

Figure 1. Assay Principle.

Related Products

1. MET-5054: L-Amino Acid Assay Kit
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5071: Taurine Assay Kit
4. MET-5136: D-Amino Acid Kit (Colorimetric)
5. MET-5196: Proline Assay Kit

Kit Components (shipped on blue ice)

1. L-Threonine Standard (Part No. 52051C): One 50 µL vial at 20 mM.
2. 10X Assay Buffer (Part No. 51962A): One 30 mL bottle.
3. ThrDH Enzyme (Part No. 52052D): One 200 µL amber vial.
4. NAD+ (Part No. 51964D): One 400 µL vial.
5. 10X Colorimetric Probe (Part No. 51946D): Two 1 mL amber vials.

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate
3. Spectrophotometric microplate reader capable of reading absorbance at 450 nm
4. 10 kD cutoff centrifugal filter units

Storage

Upon receipt, store the 10X Assay buffer at room temperature. Store all other components at -80ºC. Avoid multiple freeze/thaw cycles. The 10X Colorimetric Probe and ThrDH Enzyme are light sensitive and must be stored accordingly.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by diluting the 10X Colorimetric Probe 1:10, the ThrDH Enzyme 1:100, and the NAD+ 1:50 in 1X Assay Buffer. For example, for 20 assays, add 400 µL of the 10X Colorimetric Probe, 40 µL of the ThrDH Enzyme, and 80 µL of NAD+ to 3480 µL of 1X Assay Buffer.

  Note: Prepare only enough for immediate use by scaling the above example proportionally. Do not store diluted solutions.
- Control Mix: Prepare a Control Mix by diluting the 10X Colorimetric Probe 1:10 and the NAD+ 1:50 in 1X Assay Buffer. For example, for 20 assays, add 400 µL of the 10X Colorimetric Probe and 80 µL of NAD+ to 3520 µL of 1X Assay Buffer.

  Note: Prepare only enough for immediate use by scaling the above example proportionally. Do not store diluted solutions.

Preparation of Samples

- Cell lysates: Resuspend cells in 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant and filter the solution with a 10 kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through may be assayed undiluted or diluted as necessary into 1X Assay Buffer.

- Tissue lysates: Sonicate or homogenize tissue sample in 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant and filter the solution with a 10 kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through may be assayed directly or diluted as necessary into 1X Assay Buffer.

- Serum or Plasma: Deproteinate the sample by running it through a 10 kD cutoff centrifugal filter unit and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant as necessary into 1X Assay Buffer just prior to performing the assay.

- Urine: To remove insoluble particles, centrifuge at 10000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10 kD cutoff centrifugal filter unit to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary into 1X Assay Buffer.

Preparation of Standard Curve

Prepare fresh Threonine standards before use by diluting in deonized water according to Table 1 below.

Standard Tubes	20 mM L-Threonine Solution (µL)	Deionized Water (µL)	L-Threonine (µM)
1	5	495	200
2	250 of Tube #1	250	100
3	250 of Tube #2	250	50
4	250 of Tube #3	250	25
5	250 of Tube #4	250	12.5
6	250 of Tube #5	250	6.25
7	250 of Tube #6	250	3.1
8	0	250	0

Table 1. Preparation of L-Threonine Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one to be treated with ThrDH (+ThrDH) and one without the enzyme (-ThrDH) to measure endogenous threonine background.
2. Add 50 µL of each L-Threonine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 200 µL of Reaction Mix per well to standards and one half of the paired sample wells.
4. Add 200 µL of Control Mix per well the other half of the paired sample wells.
5. Incubate for 5 minutes at 37°C protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
6. Read the absorbance of each well on a microplate reader using 450 nm as the primary wavelength.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without ThrDH (-ThrDH) from the sample well values containing enzyme (+ThrDH) to obtain the difference. The absorbance difference is due to the enzyme ThrDH activity:

$$\Delta A = A_{(+ThrDH)} - A_{(-ThrDH)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of L-Threonine present in the sample. Only use values within the range of the standard curve.
6. To calculate the final L-Threonine concentration in the sample, take into account any prior dilutions performed.

Example of Results

The following figures demonstrate typical Threonine Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

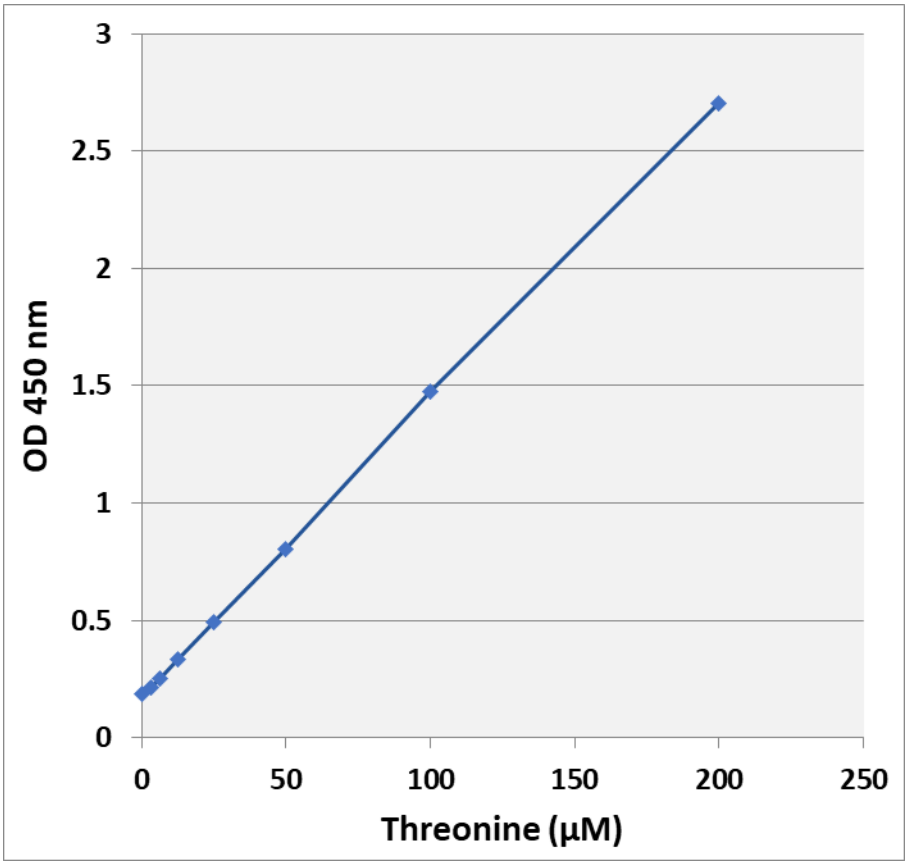

Figure 2. L-Threonine Standard Curve.

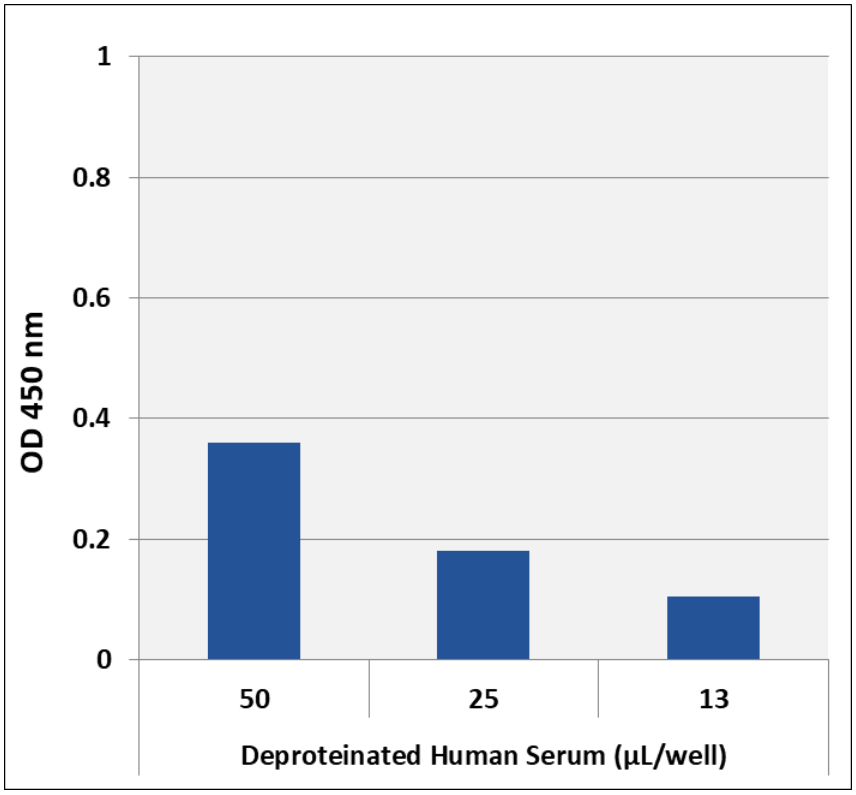

Figure 2. Detection of free Threonine in human serum. Human serum was deproteinated according to the Preparation of Samples section above.

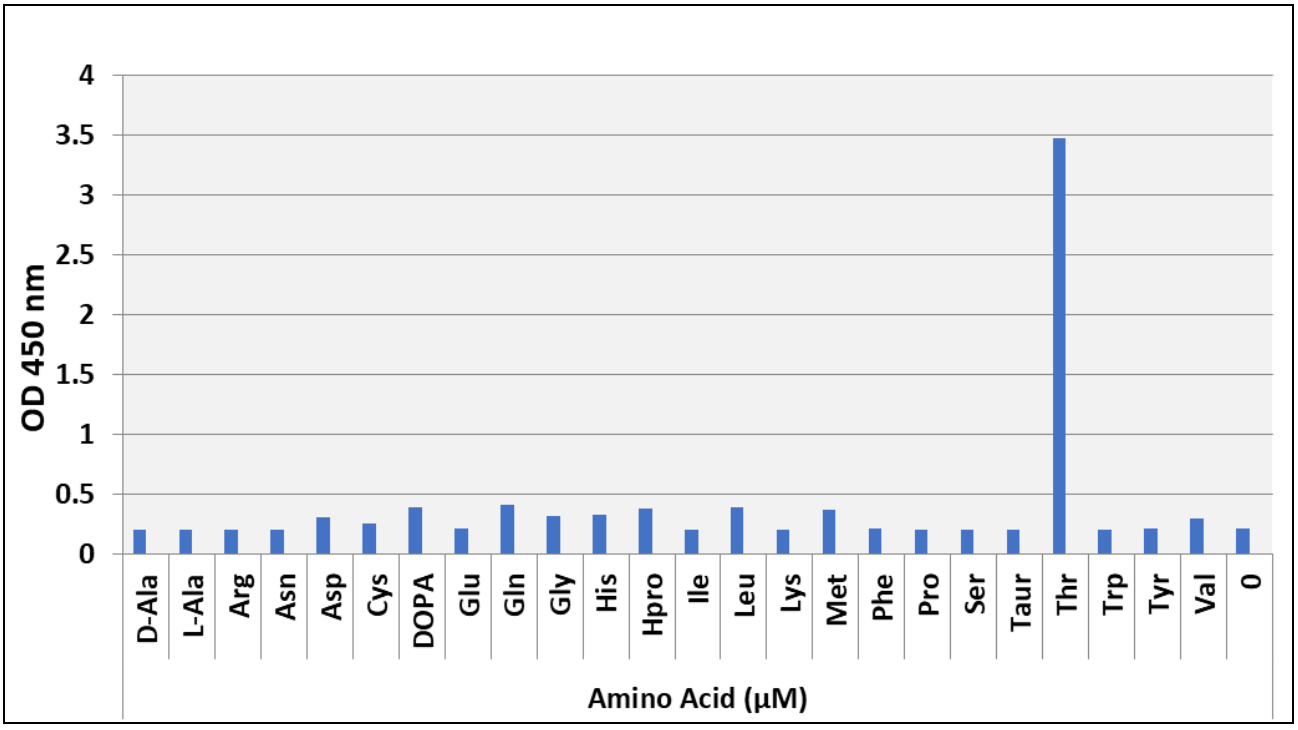

Figure 3. Specificity of L-Threonine Assay Kit. Reactions were performed in the presence of 100 µM D-Alanine (D-Ala), L-Alanine (L-Ala), L-Arginine (Arg), L-Asparagine (Asn) L-Asparate (Asp),

L-Cysteine (Cys), L-Dihydroxyphenylalanine (DOPA), L-Glutamate (Glu), L-Glutamine (Gln), L-Glycine (Gly), L-Histidine (His), L-Hydroxyproline (Hpro), L-Isoleucine (Ile), L-leucine (Leu), L-Lysine (Lys), L-Methionine (Met), L-Phenylalanine (Phe), L-Proline (Pro), L-Serine (Ser), L-Taurine (Taur), L-Threonine (Thr), L-Tryptophan (Trp), L-Tyrosine (Tyr), L-Valine (Val), or no Amino Acid Control (0).

References

1. Millar AH; Heazlewood, JL, Giglione, C, Holdsworth MJ, Bachmair, A; and Schulze, WX. (2019) *Neuroanatomical Basis of Clinical Neurology* 70: 119–151.
2. Jastrzab, R (2013). *Journal of Coordination Chemistry*. 66: 98–113
3. Dalangin R, Kim A, and Campbell RE (2020). *International Journal of Molecular Sciences*. 21: 619
4. Tang Q, Peng T Ning M, and Xi M (2021). Nutrients. 13: 2592

Warranty

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