Product Manual

Taurine Assay Kit

Catalog Number

MET- 5071 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Taurine, also known as 2-aminoethanesulfonic acid, is a small molecule found in various types of animal tissues. Taurine is a large component of bile and represents almost 0.1% of total human body weight. In contrast to most biologically occurring acids that contain the more weakly acidic carboxyl group, taurine is a sulfonic acid, and is not technically an amino acid due to its lacking the carboxyl group. Taurine is biochemically synthesized from cysteine through a series of enzymatic reactions in the pancreas: the thiol group of cysteine is oxidized to cysteine sulfinic acid by cysteine dioxygenases, cysteine sulfinic acid is decarboxylated by sulfinoalanine decarboxylase to form hypotaurine, and hypotaurine is oxidized to form taurine by hypotaurine dehydrogenase.

Taurine has many fundamental functions in mammalian biology. Taurine is conjugated to chenodeoxycholic acid and cholic acid forming the bile salts sodium taurochenodeoxycholate and sodium taurocholate. Taurine's sulfonic acid group has a low pKa which keeps this moiety negatively charged in the intestinal tract and therefore improves the detergent properties of the bile conjugate. Taurine is also important for adipose tissue and cardiovascular function as well as maturation and function of central nervous system, skeletal muscle, and the retina. Taurine has been associated with many physiological phenomena including inhibition of neurotransmission, long-term potentiation in the striatum/hippocampus, stabilization of the membrane, inhibition of neutrophil/macrophage respiratory burst, adipose tissue regulation, calcium level regulation, osmotic shock recovery, prevention of glutamate excitotoxicity, and protection from epileptic seizures.

Cell Biolabs' Taurine Assay Kit is a simple colorimetric assay that measures the total amount of free taurine present in foods or biological samples in a 96-well microtiter plate format. Taurine found in conjugated molecules (primary bile acids) is not detected. Each kit provides sufficient reagents to perform up to 200 assays*, including blanks, taurine standards, background controls and unknown samples. Sample taurine concentrations are determined by comparison with a known taurine standard. The kit has a detection sensitivity limit of 15.6 µM taurine.

Note: Each sample replicate requires 2 assays, one treated with Stop Solution and one treated with Quenching Solution. Taurine content is calculated from the difference in OD readings from the 2 wells (See Calculation of Results section on page 7).

Assay Principle

Cell Biolabs' Taurine Assay Kit measures Taurine within food or biological samples. Taurine is oxygenated by taurine dioxygenase in the presence of cofactor into 1-hydroxy-2-aminoethanesulfonic acid which decomposes to aminoacetaldehyde and sulfite. The enzymatic reaction is terminated by the addition of EDTA (an inhibitor of taurine dioxygenase). The sulfite is then detected with a highly specific colorimetric thiol probe. Samples and standards are read with a standard 96-well colorimetric plate reader. Samples are compared to a known concentration of taurine standard within the 96-well microtiter plate format (Figure 1).

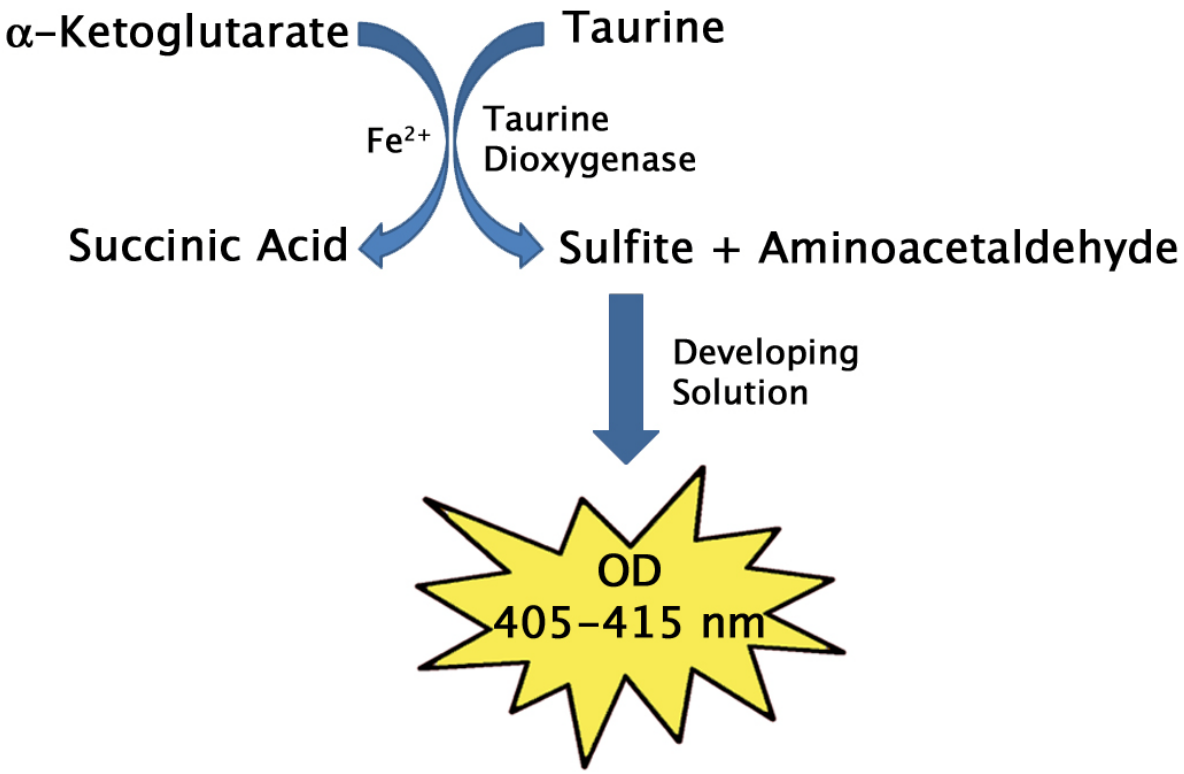

Figure 1. Taurine Assay Principle

Related Products

1. STA-631: Total Bile Acid Assay Kit (Colorimetric)
2. MET-5005: Total Bile Acid Assay Kit (Fluorometric)
3. MET-5007: Cholic Acid ELISA Kit
4. MET-5008: Chenodeoxycholic Acid ELISA Kit
5. MET-5053: Total Thiol Assay Kit (Colorimetric)
6. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
7. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
8. MET-5070: Glycine Assay Kit
9. STA-670: Homocysteine ELISA Kit
10. STA-674: Glutamate Assay Kit
11. STA-675: Hydroxyproline Assay Kit

Kit Components

Box 1 (shipped at room temperature)

1. Taurine Standard (Part No. 50711C): One 100 µL tube at 100 mM.
2. 10X Assay Buffer (Part No. 50712A): One 10 mL bottle.
3. 10X Reagent A (Part No. 50713A): One 1 mL tube.
4. 10X Reagent B (Part No. 50714A): One 1 mL amber tube.
5. Cofactor (Part No. 50715A): One amber tube containing 250 mg.
6. Stop Solution (Part No. 50717A): One 5 mL bottle.
7. Quenching Solution (Part No. 50718A): One 5 mL bottle.

Box 2 (shipped on blue ice packs)

1. 10X Taurine Dioxygenase (Part No. 50716D): One 1 mL tube containing recombinant Taurine Dioxygenase from *Escherichia coli*.
2. Developing Solution (Part No. 50719C): One 10 mL bottle.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Microcentrifuge tubes
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Standard 96-well black microtiter plate and/or cell culture microplate
7. Multichannel micropipette reservoir
8. Microplate reader capable of reading at 405-415 nm

Storage

Upon receipt, store the Taurine Standard, Taurine Dioxygenase, and Developing Solution at -20ºC. Store the remaining components at 4ºC.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer to 1X with deionized water. Stir to homogeneity. Store at room temperature.
- 100X Cofactor: Weigh out at least 6 milligrams of solid Cofactor. Resuspend by vortexing thoroughly in 1X Assay Buffer at 6 mg/mL. The 100X Cofactor will change color to bright orange over the first several minutes of resuspension, but is stable for 8 hours at room temperature. Mix

100X Cofactor thoroughly just before use in reaction mix below. Discard unused 100X Cofactor after 8 hours at room temperature.

- Reaction Mix: Prepare a Reaction Mix by diluting the 10X Reagent A 1:10, 10X Reagent B 1:10, 100X Cofactor 1:100, and Taurine Dioxygenase 1:10 in 1X Assay Buffer. For example, add 100 µL 10X Reagent A, 100 µL 10X Reagent B, 10 µL 100X Cofactor, and 100 µL of Taurine Dioxygenase to 690 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum or Plasma: Deproteinate the sample by running it through a centrifugal filter unit (For example use an Amicon Ultra 0.5 mL 10K Cat. No. UFC501024) and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed undiluted or diluted as necessary in 1X Assay Buffer.

Notes:

All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

Preparation of Standard Curve

Prepare fresh Taurine Standards before use by diluting in 1X Assay Buffer according to Table 2 below.

Standard Tubes	100 mM Taurine Solution (µL)	1X Assay Buffer (µL)	Taurine (µM)
1	5	495	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 2. Preparation of Taurine 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 sample replicate requires two paired wells, one to be treated with Stop Solution and one with Quenching Solution. While the Stop Solution contains EDTA (which inhibits Taurine Dioxygenase), the Quenching Solution contains both EDTA and a quenching reagent that specifically destroys any sulfite produced by Taurine Dioxygenase, therefore serving as a background control.

2. Add 50 µL of each Taurine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature.
4. Add 50 µL of Stop Solution to the Taurine Standards and one half of the paired sample wells.
5. Add 50 µL of Quenching Solution to the other half of the paired sample wells.
6. Add 50 µL of Developing Solution to all of the wells and mix thoroughly.
7. Incubate the plate on an orbital shaker for 3 minutes at room temperature.
8. Read the plate at 405-415 nm using a microplate spectrophotometer.

Example of Results

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

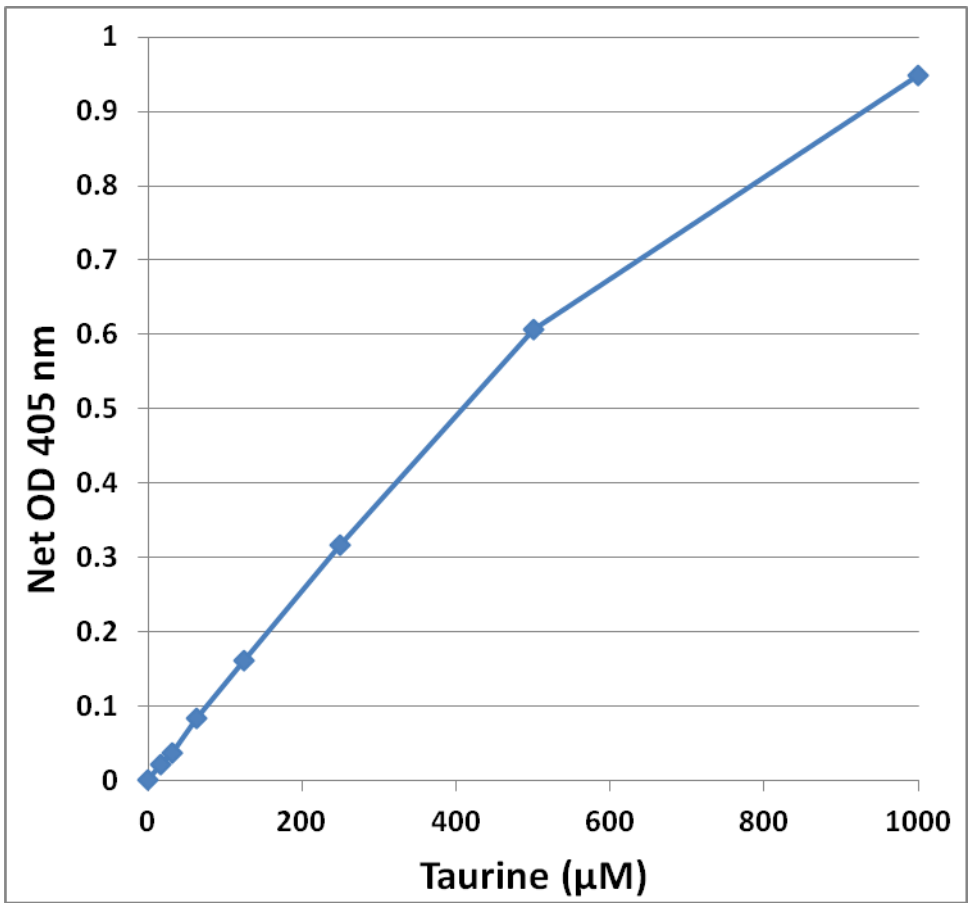

Figure 2: Taurine Standard Curve.

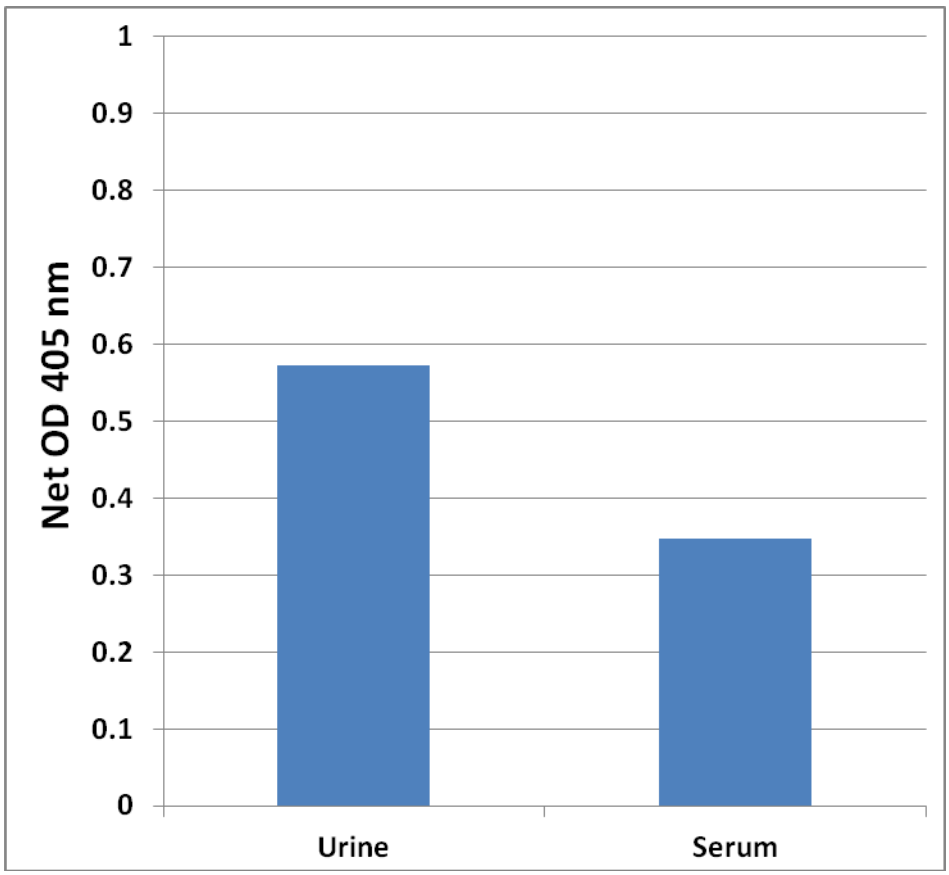

Figure 3: Taurine Detection in Human Urine or Serum. Human samples were tested undiluted according to the Assay Protocol (note: human serum was deproteinated according to the Preparation of Samples section).

Calculation of Results

1. Determine the average optical density (OD) 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 containing Quenching Solution from the sample well values containing Stop Solution to obtain the difference. The OD difference is due to the enzyme Taurine Dioxygenase activity (see Figure 3):

$$\text{Net OD} = OD_{Stop} - OD_{Quenching}$$

5. Compare the Net OD of each sample to the standard curve to determine and extrapolate the quantity of taurine present in the sample. Only use values within the range of the standard curve.

References

1. Schuller-Levis GBm Park E (2003). *FEMS Microbiol. Lett.* **226**: 195–202.
2. Sumizu K (1962). *Biochim. Biophys. Acta.* **63**: 210–212.
3. Huxtable, RJ (1992). *Physiol Rev.* **72**: 101–63.

4. Irving CS, Hammer BE, Danyluk SS, Klein PD (1980). *J. Inorg. Biochem*. **13**: 137–50.
5. Olive, MF (2002). *Amino Acids*. **23**: 345–57.
6. Dominy, J Jr; Thinschmidt, JS; Peris, J; Dawson, R Jr; Papke, RL (2004). *J. of Neurochem*. **89**: 1195–205.
7. Foos, TM; Wu, JY (2002). *Neurochem. Res*. **27**: 21–6.
8. Stummer W, Betz AL, Shakui P, Keep RF (1995). *J. Cerebr. Blood Flow Metab*. **15**: 852–9.
9. Leon R, Wu H, Jin Y, Wei J, Buddhala C, Prentice H, Wu JY (2008). *J. Neurosci. Res.* **87**: 1185–1194.
10. El Idrissi A, Messing J, Scalia J, Trenkner E; Messing; Scalia; Trenkner (2003). *Adv. Exp. Med. Bio.* **526**: 515–25.

Warranty

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