

**Instructions for Use**

Version: 1.1.1

Revision date: 22-Jul-24

**Taurine Assay Kit****Catalog No.:** abx298951**Size:** 100 Assays**Storage:** Store all components at 4°C.**Application:** For quantitative detection of Taurine concentrations in tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.**Detection Range:** 0.1 mmol/L – 10 mmol/L

**Introduction:** Taurine is a sulfonic amino acid, an essential amino acid in the central nervous system and skeletal muscle development in humans and many other species. Taurine is abundant in the brain, heart, breast, gall bladder and kidney and has important roles in health and disease in these organs throughout the lifespan. Taurine has many diverse biological functions, including in metabolism, serving as a neurotransmitter in the brain, a stabilizer of cell membranes and a facilitator in the transport of ions such as sodium, potassium, calcium and magnesium. Taurine is highly concentrated in animal and fish protein, which are good sources of dietary Taurine.

Abbexa's Taurine Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Taurine concentrations. The dye reagents react with Taurine to create an absorption maximum at 400 nm. The intensity of the color is proportional to the concentration of Taurine, which can then be calculated.

**Kit components**

1. 96 well microplate
2. Assay Buffer 1: 3 × 30 ml
3. Assay Buffer 2: 30 ml
4. Dye Reagent A: 10 ml
5. Dye Reagent B: 0.6 ml
6. Standard: 1 vial
7. Plate sealer: 3

**Materials Required But Not Provided**

1. Microplate reader (400 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Mortar
7. Convection Oven
8. Sonicator
9. Water bath

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## Protocol

### A. Preparation of Sample and Reagents

#### 1. Reagents

- **Dye Reagent Working Solution**

Add 0.6 ml of Dye Reagent B into the Dye Reagent A vial and mix thoroughly. If any precipitates are observed, warm the vial using a water bath until the precipitates have dissolved.

- **Standard Solution**

Add 1 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 0.5 ml of this solution and add 0.5 ml of distilled water to prepare the Standard Solution (concentration 10 mmol/L). Unused Standard Solution can be stored at 4°C.

#### 2. Sample

- **Cell and Bacterial samples**

Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 0.75 ml of Assay Buffer 1 for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Add 0.25 ml of Assay Buffer 2 and centrifuge at 10,000 × g for 10 minutes. Transfer the supernatant to a new tube, then analyze immediately.

- **Tissue samples**

Homogenize 0.1 g of sample in 0.75 ml of Assay Buffer 1, then incubate at 40°C for 30 minutes. Add 0.25 ml of Assay Buffer 2, then centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube and analyze immediately.

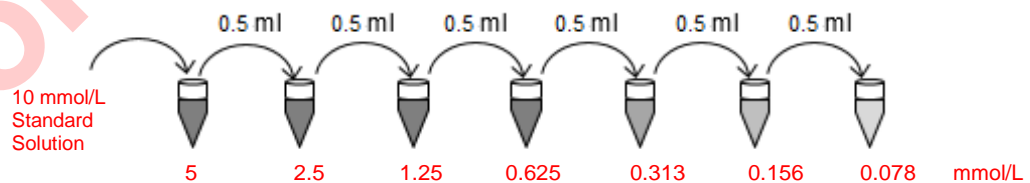
- **Serum or plasma samples**

To 0.5 ml of serum or plasma sample, add 0.75 ml of Assay Buffer 1, then centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, then add 0.25 ml of Assay Buffer 2. Mix thoroughly, then analyze immediately.

### B. Assay Procedure

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 5 mmol/L, 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.313 mmol/L, 0.156 mmol/L, and 0.078 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 10 mmol/L Standard Solution to the 1<sup>st</sup> tube and mix thoroughly. Transfer 0.5 ml from the 1<sup>st</sup> tube to the 2<sup>nd</sup> tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 100 µl of sample to the sample wells.
4. Add 100 µl of prepared standards to the standard wells.
5. Add 100 µl of distilled water to the blank wells.
6. Add 100 µl of Dye Reagent Working Solution to all wells.
7. Tap the plate gently to mix.
8. Incubate in a convection oven at 90°C for 15 minutes.
9. Allow to cool, then read and record absorbance at 400 nm.

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### C. Calculations

Taurine concentration per g of sample:

$$\text{Taurine } (\mu\text{mol/g}) = \frac{C_{\text{Standard}} \times V_{\text{Standard}} \times V_{\text{Assay}}}{V_{\text{Sample}} \times W} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}} = \frac{10}{W} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}}$$

Taurine concentration per 10<sup>4</sup> cells or bacteria:

$$\text{Taurine } (\mu\text{mol}/10^4 \text{ cells}) = \frac{C_{\text{Standard}} \times V_{\text{Standard}} \times V_{\text{Assay}}}{V_{\text{Sample}} \times N} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}} = \frac{10}{N} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}}$$

Taurine concentration per ml serum or plasma:

$$\text{Taurine } (\mu\text{mol/ml}) = 3 \times \frac{C_{\text{Standard}} \times V_{\text{Standard}}}{V_{\text{Sample}}} \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}} = 30 \times \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Standard}} - OD_{\text{Blank}}}$$

where:

<b>C<sub>Protein</sub></b>	Concentration of protein (in mg/ml)
<b>C<sub>Standard</sub></b>	Concentration of highest standard (10 mmol/L= 10 μmol/ml)
<b>W</b>	Weight of the sample (in g)
<b>N</b>	Number of cells or bacteria (× 10 <sup>4</sup> )
<b>V<sub>Assay</sub></b>	Volume of Assay Buffer 1 and Assay Buffer 2 (1 ml)
<b>V<sub>Sample</sub></b>	Volume of sample (0.1 ml)
<b>V<sub>Standard</sub></b>	Volume of standard (0.1 ml)