Taurine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1249

(Version 1.5A)

Detection and Quantification of Taurine Content in Tissue extracts, Cell lysate and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Taurine is a sulfur amino acid, an essential amino acid in pre-term and newborn infants of humans and many other species. Taurine is abundant in the brain, heart, breast, gallbladder and kidney and has important roles in health and disease in these organs. Taurine has many diverse biological functions 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. Taurine has many important metabolic roles. Supplements can stimulate prolactin and insulin release.

Taurine Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of taurine in a variety of samples. Taurine reacts with acetylacetone to form a yellow complex. The intensity of the product color, measured at 400 nm, is proportional to the taurine concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 3	4 °C
Assay Buffer II	30 ml x 1	4 °C
Dye Reagent A	10 ml x 1	4 °C
Dye Reagent B	0.6 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 400 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Hot air circulation oven

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 20 mmol/L of standard stock solution, store at 4 °C for 1 month after reconstitution. Then dilute to 10 mmol/L standard top solution by adding 0.5 ml stock solution into 0.5 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Dye Reagent Working Solution: Add 0.6 ml Dye Reagent B into Dye Reagent A. Prepare Working Solution for immediate use or keep in dark at -20 °C for 1 week.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 0.75 ml Assay Buffer I for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); then add 0.25 ml Assay Buffer II, centrifuged at 10,000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 0.75 ml Assay Buffer I, incubate at 40 °C water bath for 30 minutes; then add 0.25 ml Assay Buffer II, centrifuged at 10,000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For serum or plasma samples

Add 0.75 ml Assay Buffer I into 0.5 ml samples, mix; centrifuged at 10,000g for 10 minutes, take the supernatant into a new centrifuge tube, then add 0.25 ml Assay Buffer II, mix.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Dye Reagent Working Solution	100 µl	100 µl	100 µl
Mix, cover the plate adhesive strip, put the plate into the hot air circulation oven, 90 °C for 15 minutes. When cold, record absorbance measured at 400 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

** If any precipitate, please add the samples into the microcentrifuge tube, centrifuged at 10,000g for 10 minutes; then add the supernatant into the microplate to read the result.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \quad (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the quantity of cells or bacteria

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V \times V_{Sample} / (V + V_{Assay})} \quad (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer I and Assay Buffer II, μl

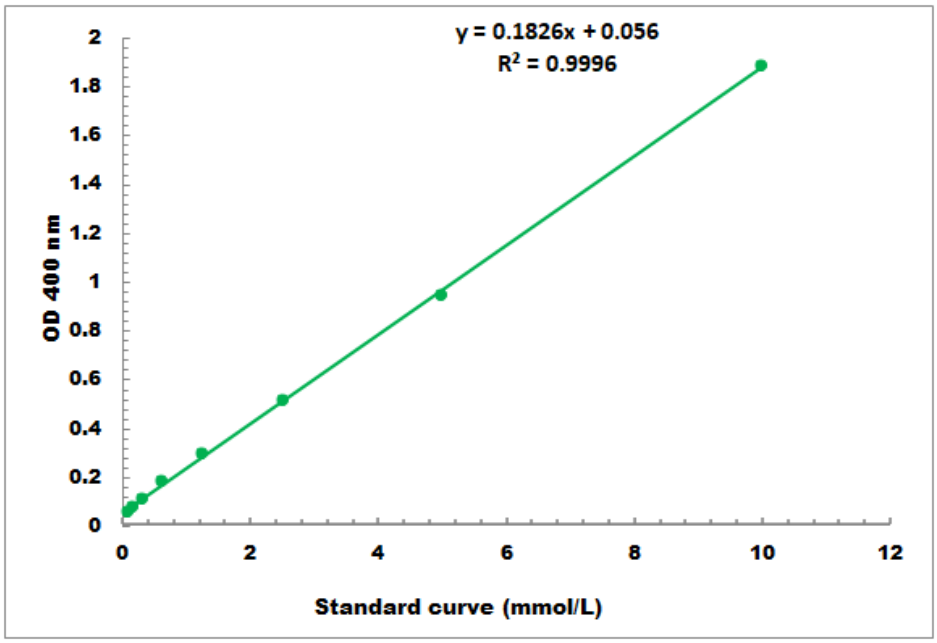
W: the weight of sample, g

V: the volume of sample in sample preparation, ml

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L