Taurine Assay Kit (Colorimetric)

(Catalog # K988-100; 100 assays, Store kit at -20°C)

10/16

I. Introduction:

Taurine is a sulfur-containing amino acid with various beneficial roles in biological processes, such as, calcium flux and neuronal excitability, osmoregulation, detoxification and membrane stabilization. It is synthesized in cells as an end product of cysteine metabolism. Taurine level in urine is inversely proportional to the risk factor in cardiovascular diseases. Emerging beneficial effects of Taurine in Type 1 and Type 2 diabetes model establishes its effectiveness as a therapeutic molecule. Therefore, determination of Taurine levels in biological samples is an important tool for research in disease diagnostics and molecular therapeutics. BioVision's Taurine Assay Kit enables the measurement of Taurine level in both biological fluids as well as food products, such as energy drinks. The kit utilizes the ability of an enzyme to convert Taurine into aminoacetaldehyde and sulfite. The produced sulfite is measured using a probe which can be detected using a microplate reader (OD 415 nm). This assay kit is simple, high-throughput compatible and can detect as low as 5 nmols of Taurine.

II. Applications:

- Detection of Taurine in Urine and other body fluids
- Detection of Taurine in energy drinks and other food extracts

III. Sample Type:

- Urine
- Energy drinks

IV. Kit Contents:

Components	K988-100	Cap Code	Part Number
Taurine Assay Buffer (4X)	30 ml	NM	K988-100-1
Sulfite Probe Buffer	6 ml	NM	K988-100-2
Enzyme Cofactor	2×3.6 mg	Clear	K988-100-3
Ascorbic Acid (200 mM)	1.5 ml	White	K988-100-4
Enzyme Mix	2×1 ml	Green	K988-100-5
Taurine (5 mM)	1 ml	Yellow	K988-100-6
Sulfite Probe	3×5 mg	Red	K988-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well absorbance microplate reader

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay**.**

- **Taurine Assay Buffer (4X):** Dilute the buffer to 1X using dH_2O (i.e. mix 2 ml of Taurine Assay Buffer with 6 ml of dH_2O to make 8 ml 1X Assay Buffer). Store at 4 °C or -20 °C. Bring to room temperature before use.
- **Sulfite Probe Buffer**: Ready to use. Store at 4 °C or -20 °C. Bring to room temperature before use.
- **Enzyme cofactor:** Keep at -20ºC. Before use, add 100 µl dH_2O to one vial, mix well. Avoid aerial exposure. Once reconstituted, use within 1 week.
- **Ascorbic acid**: Ready to use. Aliquot and store at -20°C. Avoid aerial exposure and multiple freeze thaw cycle. Use aliquot within a week after thawing.
- **Enzyme mix**: Ready to use. Aliquot and Store at -20°C. Avoid multiple freeze/thaw of the enzyme. Use within 3 months.
- **Taurine**: Ready to use. Store at -20°C or 4ºC. Thaw before use.
- **Sulfite Probe**: Store at -20°C. Before use, add 1.4 ml of Sulfite Probe Buffer to each tube and reconstitute the lyophilized probe by gentle pipetting, wait until it is completely dissolved. Store at –20°C, stable for 2 months.

VII. Taurine Assay Protocol:

1. **Sample Preparation:** Centrifuge urine sample at 14000 g for 10 mins at 4 ºC. Take the supernatant and filter through a 10k MWCO filter 10 kDa Spin Filter (Catalog no. 1997). The filtered urine sample is ready to be assayed. Add 5-25 µl of filtered urine into desired well(s) in a clear 96-well plate. Before experiment, prepare 2 ml **Working Solution** (WS): add 2 µl of Enzyme cofactor solution and 15 µl of Ascorbic acid to 2 ml of Taurine assay buffer and mix well. Always prepare fresh WS. **Use the WS within an hour after being prepared**. Energy drinks: samples can be used directly. If necessary, dilute the sample with water. For Background Control (BC), add similar amount of sample in separate wells. Then adjust the final volume to 180 µl in all wells using WS.

Notes:

a. Taurine concentration varies over a wide range depending on the sample. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

b. For samples having high protein content, we recommend deproteinizing the samples (tissue or cell lysate or biological fluids) using 10 kDa Spin Column (Cat. # 1997 or equivalent). Add sample to the spin column, centrifuge at 10,000 X g, 4°C for 10 min. collect and use the filtrate.

c. To ensure accurate determination of Taurine in the test samples or for samples having low concentrations of Taurine, we recommend spiking samples with a known amount of Taurine Standard (e.g. 15 nmol).

d. Thiol present in biological samples might show high background signal. To quantify the signal contribution from taurine-generated sulfite only, add formaldehyde to 5 mM final concentration *before adding the probe* (step 4). This will complex with the sulfite and prevent signal generation. The difference of signal (with and without formaldehyde) will correspond to the signal from sulfite only.

2. **Taurine Standard Curve Preparation:** Add 0, 2, 4, 6, 8 and 10 µl of 5 mM Taurine into a series of wells in a 96-well plate and adjust the final volume to 180 µl/well with WS to generate 0, 10, 20, 30, 40 and 50 nmol/well of Taurine Standards respectively.

   Note: For unknown samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range.

3. **Enzyme mix:** To each well of Sample(s) and Taurine Standards, add 20 µl of Enzyme Mix, mix properly, and then incubate the 96 well plate at 30ºC for 30 mins. For BC, replace Enzyme mix with 20 µl of Taurine Assay Buffer.

4. **Sulfite Probe**: add 30 µl of the Sulfite Probe to each well containing Sample, Background Control, and Taurine Standards Mix well, incubate for 5 minutes.

5. **Measurement:** Measure absorbance in an endpoint mode at 415 nm using a microplate reader.

6. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Taurine-Standard Curve and obtain the slope of the curve (ΔAbsorbance/nmol). If the background control reading is significant then subtract the background control reading from sample reading.

$$[\mathbf{Taurine}] = \mathbf{B\ X\ D/V} = \mathbf{nmol/ml}$$

Where:

B = Taurine in sample based on Std. curve slope (nmol)
V = the sample volume added into the reaction well (ml)
D = Sample dilution factor (D=1 when samples are undiluted)

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

$$\textbf{For spiked samples Taurine amount in sample well} = \frac{(\mathbf{OD_{Sample\ corrected}})}{(\mathbf{OD_{Sample+Tau\ Standard}})-(\mathbf{OD_{Sample\ (corrected)}})} * \text{Taurine spike (nmole)}$$

Taurine Molecular weight:125.15.

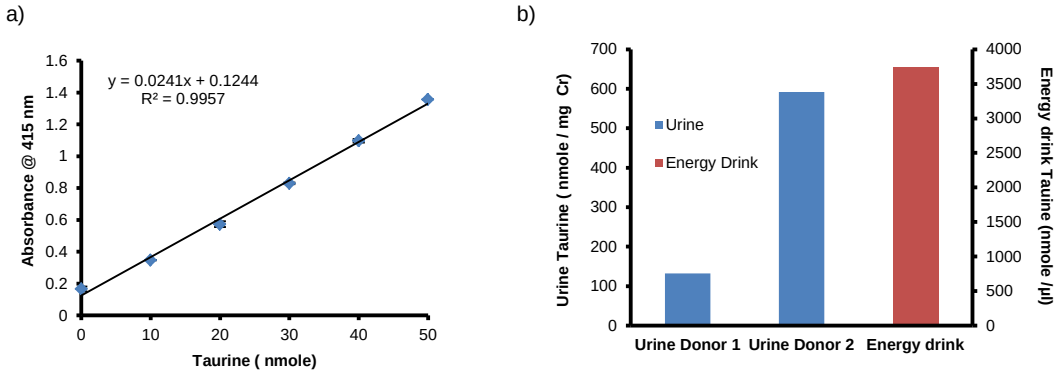

Figure: (a) **Taurine Standard Curve** (5 nmoles-25 nmoles), error bars indicate SD (n=3). (b) **Taurine in urine and energy drink**: Taurine present in Donor 1 urine sample was 134 nmoles/ mg Creatinine (Cr) and in Donor 2 was 592 nmoles/ mg Creatinine. Energy drink was diluted 10x with water before performing the assay.

VIII. RELATED PRODUCTS:

Taurine Dioxygenase (P1071)
Cysteine Assay Kit (Fluorometric) (K558)
Glycine Assay Kit (Fluorometric) (K589)
Glutamate Colorimetric Assay Kit (K629)
Sarcosine Colorimetric/Fluorometric Assay Kit (K636)
Albumin (Albuminuria) Fluorometric Assay Kit (K550)
Creatinine Colorimetric/Fluorometric Assay Kit (K627

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