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Product Information

Taurine Assay Kit

Catalog Number **MAK355**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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 models 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.

The Taurine Assay Kit enables the measurement of taurine levels 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 amino-acetaldehyde and sulfite. The produced sulfite is measured using a probe which can be detected using a microplate reader (A_{415}). This assay kit is simple, high-throughput compatible, and can detect as low as 5 nmols of taurine.

The kit is suitable for the detection of taurine in urine and other body fluids, as well as energy drinks and other food extracts.

Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

Taurine Assay Buffer (4×) Catalog Number MAK355A	30 mL
Sulfite Probe Buffer Catalog Number MAK355B	6 mL
Enzyme Cofactor Catalog Number MAK355C	2 × 3.6 mg
Ascorbic Acid (200 mM) Catalog Number MAK355D	1.5 mL
Enzyme Mix Catalog Number MAK355E	2 × 1 mL
Taurine (5 mM) Catalog Number MAK355F	1 mL
Sulfite Probe Catalog Number MAK355G	3 × 5 mg

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Clear flatbottom 96 well plates
- Spectrophotometric multiwell plate reader, capable of 30 °C temperature setting
- Refrigerated microcentrifuge capable of RCF ≥14,000 × *g*
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Taurine Assay Buffer (1×): Dilute the Taurine Assay Buffer (4×) 4-fold with ultrapure water (i.e., mix 2 mL of Taurine Assay Buffer (4×) with 6 mL of ultrapure water). Store at 4 °C or –20 °C. Bring to room temperature prior to use.

Sulfite Probe Buffer: Ready to use. Bring to room temperature prior to use.

Enzyme Cofactor: Prior to use, reconstitute by adding 100 µL of ultrapure water to one vial, mix well. Avoid exposure to air. Once reconstituted, use within 1 week.

Ascorbic Acid: Ready to use. Aliquot and store at –20 °C. Avoid exposure to air and multiple freeze/thaw cycles. Use aliquot within 1 week after thawing.

Enzyme Mix: Ready to use. Aliquot and Store at –20 °C. Avoid multiple freeze/thaw cycles.

Taurine: Ready to use. Bring to room temperature prior to use.

Sulfite Probe: Reconstitute vial by adding 1.4 mL of Sulfite Probe Buffer. Gently pipette up and down and wait until completely dissolved prior to use. Stable for 2 months once reconstituted.

Working Solution: **Prepare fresh and use within an hour after being prepared**. Prepare Working Solution by adding:

2 µL of Enzyme Cofactor
15 µL of Ascorbic Acid
2 mL of Taurine Assay Buffer

Mix well.

Procedure

Notes: Taurine concentration varies over a wide range depending on the sample. For unknown samples, it is suggested to perform a pilot experiment by testing several sample volumes to ensure the readings are within the Standard Curve range.

- For samples having high protein content, we recommend deproteinizing the samples (tissue or cell lysate, or biological fluids) using a Corning Spin-X UF concentrator. Add sample to the spin column, centrifuge at 10,000 × *g*, for 10 minutes at 4 °C. Collect and use the filtrate.
- To ensure accurate determination of taurine in the test samples or for samples having low concentrations of taurine, it is suggested to spike samples with a known amount of Taurine Standard (e.g., 15 nmol).
- Thiol present in biological samples might show a high background signal. To quantify the signal contribution from taurine-generated sulfite only, add formaldehyde at a 5 mM final concentration before adding the probe. 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.

Sample Preparation

Urine: Centrifuge urine sample at 14,000 × *g* for 10 minutes at 4 °C. Take the supernatant and filter through a Corning Spin-X UF concentrator. 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. For Background Control (BC), add similar amount of sample in separate wells, then adjust the final volume to 180 µL in all wells using Working Solution.

Energy drinks: Samples can be used directly. If necessary, dilute the sample with ultrapure 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 Working Solution.

Standard Curve Preparation

Prepare Taurine Standards in desired wells of a clear 96 well plate according to Table 1. Mix well.

Table 1.
Preparation of Taurine Standards

Well	5 mM Taurine	Working Solution	Taurine (nmol/well)
1	0 µL	180 µL	0
2	2 µL	178 µL	10
3	4 µL	176 µL	20
4	6 µL	174 µL	30
5	8 µL	172 µL	40
6	10 µL	170 µL	50

Assay Reaction

1. To each well of Sample(s) and Taurine Standards, add 20 µL of Enzyme Mix, mix well.
2. For Background Control well(s), add 20 µL of Taurine Assay Buffer.
3. Incubate the 96 well plate at 30 °C for 30 minutes.
4. Add 30 µL of the Sulfite Probe to each well containing Sample, Background Control, and Taurine Standards. Mix well then incubate for 5 minutes at 30 °C.

Measurement

Measure absorbance at 415 nm (A_{415}) in endpoint mode using a microplate reader.

Results

1. Subtract 0 Standard reading from all readings.
2. Plot the Taurine Standard Curve and obtain the slope of the curve (ΔA_{415}/nmol).
3. If the background control reading is significant then subtract the background control reading from sample reading.

$$\text{Taurine (nmol/ mL)} = (B \times D) / V$$

where:
B = Taurine in sample based on Std. curve slope (nmol)
V = sample volume added into the reaction well (mL)
D = sample dilution factor (D = 1 for undiluted samples)

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

For spiked samples Taurine amount in sample well =

$$\frac{A_{415}\ Corrected\ Sample}{(A_{415}\ Sample + Taurine\ Std.) - (A_{415}\ Corrected\ Sample)} \times \textit{Taurine Spike (nmol)}$$

Taurine Molecular Weight: 125.15 g/mol

Figure 1.
Typical Taurine Standard Curve

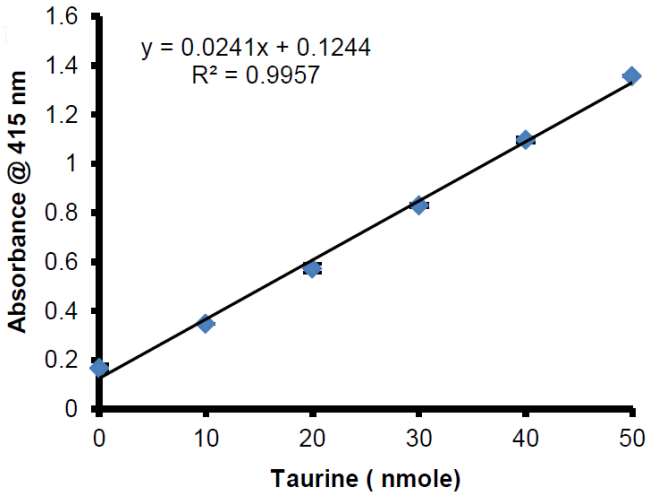
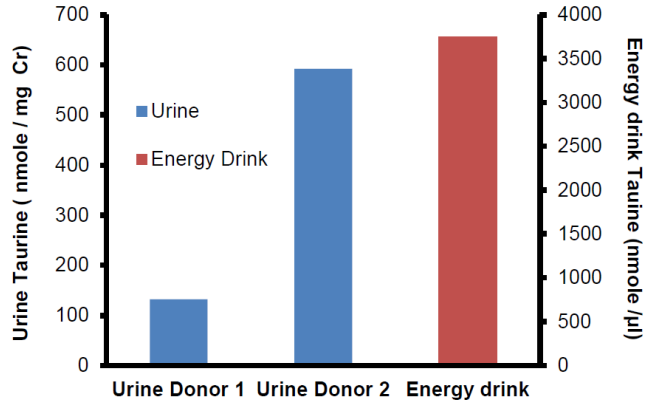


Figure 2.
Taurine in Urine and Energy Drink



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 10-fold with ultrapure water before performing the assay.

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