Taurine Assay Kit

ARG83369 Taurine Assay Kit is an Assay kit for the quantification of Taurine in plasma, serum, urine, cell lysate and tissue lysate

Catalog number: ARG83369

Package: 200 assays

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PRINCIPLE OF THE ASSAY

ARG83369 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 (use as inhibitor of taurine dioxygenase). The sulfite is then detected with a highly specific colorimetric thiol probe. Samples and standards are measured at a wavelength of 405nm ±2nm. The concentration of total Taurine in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, the Taurine Standard, 10X Taurine Dioxygenase, Developing Solution should store at-20°C and avoid repeated freeze-thaw cycles. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
Taurine Standard (100mM)	100 µl	-20°C
Cofactor (250 mg)	1 vial	4°C
10X Assay Buffer	10 mL	4°C
10X Reagent A	1 mL	4°C
10X Reagent B	1 mL	4°C
10X Taurine Dioxygenase	1 mL	-20°C
STOP solution	5 mL (Ready-to-use)	4°C
Quenching Solution	5 mL (Ready-to-use)	4°C
Developing Solution	10 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405nm.
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- Microplate shaker.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, the Taurine Standard, 10X Taurine Dioxygenase, Developing Solution should store at-20°C and avoid repeated freeze-thaw cycles. Store all other components at 4°C. Use the kit before expiration date.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue Lysates: Sonicate or homogenize tissue sample in cold 1X PBS or 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Cell Lysates: Wash cells 3 times with cold 1X PBS prior to lysis. Lyse cells with sonication or homogenation in cold 1X PBS or 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2,000 x g and 4°C for 10 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer. Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Urine: To remove insoluble particles, centrifuge at 10,000 x g for 5 minutes at 4°C. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer

REAGENT PREPARATION

- **1X Assay Buffer**: Dilute **10X Assay Buffer** into distilled water to yield 1X Assay Buffer, mix well. Storage at 2-8°C.
- **100X Cofactor:** Weigh out at least 6 milligrams of Cofactor. Add **1X Assay Buffer** to reconstitute the lyophilized Cofactor. The color of 100X Cofactor will change to bright orange over the first several minutes of resuspension. The 100X Cofactor is stable for 8 hours at room temperature. DO NOT used the 100X Cofactor after 8 hours.
- **Reaction Buffer:** Prepare the Reaction Reagent by diluting the kit components accordingly: 10X Reagent A at 1:10, 10X Reagent B at 1:10, 100X Cofactor at 1:100, and Taurine Dioxygenase at 1:10 in 1X Assay Buffer. For example, add 100 µL of 10X Reagent A, 100 µL of 10X Reagent B, 10 µL of 100X Cofactor, and 100 µL of Taurine Dioxygenase to 690 µL of 1X Assay Buffer for a total of 1 mL Reaction Mix. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4°C.
- **Sample**: The samples can be diluted with 1X Assay Diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended making series dilutions with Assay Diluent for each unknown sample to do pre-test to determine the suitable dilution factor).

- **Taurine standard:** Prepare a series dilution of Taurine standards with 1X Assay Diluent. The **1X Assay Diluent serves as zero standard (0 pg/ml)**, and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	Taurine (µM)	1X Assay Diluent (µL)	Standards (µL)
S1	1000	594	6
S2	500	300	300 µl (S1)
S3	250	300	300 µl (S2)
S4	125	300	300 µl (S3)
S5	62.5	300	300 µl (S4)
S6	31.25	300	300 µl (S5)
S7	16.625	300	300 µl (S6)
S0	0	300	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add 50 µl of the Standards and samples into the appropriate wells.
3. Add 50 µl of the Reaction Buffer to each well, incubate for 30 minutes at RT on a microplate shaker.
4. Add 50 µl of Stop Solution to the 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 each well, incubate for 3 minutes at RT on a microplate shaker.
7. Read the OD with a microplate reader at 405 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. 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

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

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Assay Range

15.6- 1000 µM