Tribo™ Urea Colorimetric Assay (Catalog# TBS2201)

DESCRIPTION

Urea (carbamide, carbonyl diamide) is a predominant final metabolite of nitrogenous compounds in mammals, accounting for 80-90% of nitrogen excretion in humans and animals. Formed exclusively in the liver, urea is mostly transported by the bloodstream to the kidneys, where it is excreted into the urine. It is an indicator of liver and kidney functions. Determination of urea concentration is an essential task in clinical laboratories and research.

The Tribo™ Urea Colorimetric Assay provides a rapid, sensitive method to measure urea concentration in biological samples. The intensity of the color is directly proportional to the urea concentration in the sample. The assay can be performed in a convenient 96-well or 384-well microplate format and can be easily adapted to automation without a separation step.

APPLICATION

- **Direct Assay:** of urea in serum, plasma, urine, milk, cell/tissue culture, animal products and wine.
- **Drug Discovery and Screening:** effects of drugs on urea metabolism.

KIT CONTENTS (for 200 assay)

Component	Amount
Component A: Urea Standard	**1 mL (50 mg/dL)**
Component B: Urease Mix	**(50kU/L) 20 mL**
Component C: Assay Buffer	**20 mL**

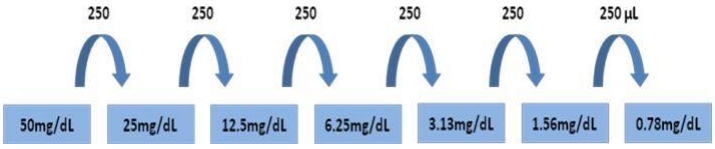
Storage conditions Store the kit at 4°C. For long-term storage, the kit should be stored at –20°C protected from light. Shelf life: 12 months.

FEATURES

- **Sensitive and Accurate:** The kit detects concentrations as low as 10µM in solution.
- **Simple and High-Throughput:** The procedure is easily adapted to automation with no separation required as a high-throughput assay.

PROCEDURES

1. Bring all reagents to room temperature (18-25˚C) before use.
2. Standard: Set up 7 points of Urea standard concentrations: 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/dL as shown in the diagram below.
3. Label 7 tubes of 1.5ml from 1-7: Add 250 µL of deionized water to tubes #2-7. Don't add water to Tube#1.
4. Add 500 µL of Urea standard (50mg/dL, Compound A) into tube #1. Standards #2-7 are then prepared by performing a 1:2 dilution from preceding standard. Mix each tube thoroughly before the next transfer. For example, to make Standard #2, remove 250 µL of Standard #1 and add it to tube #2 and mix, and so on. The deionized water serves as the zero standard (0 mg/dL).
5. Add 10 µL water, urea standard, and samples in duplicate into separate wells.
6. Add 100 µL Urease Mix (Component B) to each well. Mix thoroughly and carefully so as not to create foaming in the well.
7. Incubate 10 minutes at 37°C
8. Add 100 µL of Assay Buffer (Component C) to each well. Mix thoroughly. Incubate 30 minutes at at 37°C.
9. Read the plate at 580 -630 nm and record data.



CACULATION OF DATA

Urea concentration (mg/dL) of the sample is calculated as the formula below:

The OD values from the wells with urea reactions subtract the OD values from Block.
Conversions: 1mg/dL =167 µM =0.001% =10 ppm

Materials needs but not supplied: 96-wel microplate; Fluorescence plate reader.

REFERENCES

1. Patton CJ, Crough SR (1977): Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of urea. Anal. Chem. 49: 464-469.
2. Greenan, NS, Mulvaney RL and Sims GK (1997): A microscale method for colorimetric determination of urea in soil extracts. Communications in Soil Science and Plant Analysis. 26: 2519-2529.

RELATED PRODUCTS:

Protein Assay Kits (#TBS2005)
Cytochrome C Oxidase Activity Assay(TBS2115)
Tryptase Activity Assay(TBS2101)
Cell Nuclear Extract kit(#TBS6025)

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