Urea Assay Kit II (BA0230)

Product Datasheet | SKU BA0230

Description

A quantitative colorimetric urea (BUN) assay based on urease-catalysed conversion of urea to ammonium and carbon dioxide. The pH of the reaction medium is monitored by a chromogen and the intensity of the reaction product at 557 nm is directly proportional to the urea concentration in the sample.

Specifications

Detection Method	Colorimetric determination at OD557 nm (550-565 nm)
Detection Range	1 mg/dL (0.17 mM) to 100 mg/dL (17 mM) urea (20 µL sample) in a 96-well plate assay
Sample Type	Biological samples (e.g. plasma, serum, urine) and food/beverage samples (e.g. milk)
Species Reactivity	All
Assay Time	5 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped at room temperature. Store Reagent and Standard at 4°C upon receiving. Urease can be stored from -20°C to 4°C. For long-term storage, keep Standard at -20°C.
Shelf Life	6 months after receipt.
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Reagent	10 mL	4°C
Standard (200 mg/dL)	1 mL	4°C (long-term -20°C)
Urease	120 µL	-20°C to 4°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0230	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Sample preparation: serum and plasma can be assayed directly after centrifuging to remove particulates (n=1). Milk should be cleared by mixing 600 µL milk with 100 µL 6 N HCl, centrifuging 5 min at 14,000 g, transferring 300 µL supernatant into a clean tube, neutralising with 50 µL 6 N NaOH and diluting the neutralised supernatant 5-fold in distilled water (n=6.8). Urine: dilute 50-fold in distilled water (n=50); urine samples do not require an internal standard.
2. Reagent preparation: vortex reagent or warm in a bath if there are particulates. Equilibrate Reagent to room temperature. Briefly centrifuge other tubes before use.
3. Samples require an internal standard and need three separate reactions: (1) sample plus standard, (2) sample alone and (3) sample blank. For sample plus standard, add 5 µL 200 mg/dL urea and 20 µL sample. For sample and sample blank wells, add 5 µL dH2O and 20 µL sample.
4. Prepare enough Working Reagent (WR) for all samples-plus-standards and samples-alone: for each reaction combine 85 µL Reagent and 1 µL Urease.

5. Add 80 µL WR to each sample-plus-standard and sample-alone well. Add 80 µL Reagent (no Urease) to each sample blank well. Tap plate to mix briefly and thoroughly. Incubate 5 minutes at room temperature.
6. Read OD557nm (550-565 nm).

Calculation

[Urea] = [(R_Sample – R_Blank) / (R_Standard – R_Sample)] × [Standard]/4 × n (mg/dL) = 50 × [(R_Sample – R_Blank) / (R_Standard – R_Sample)] × n (mg/dL), where R_Sample, R_Blank and R_Standard are OD readings of the Sample, Sample Blank and Sample plus Standard, and n is the sample dilution factor. The internal standard volume is 4× lower than the sample volume, so its concentration is divided by 4. Conversions: BUN (mg/dL) = [Urea] / 2.14; 1 mg/dL urea equals 167 µM, 0.001% or 10 ppm. If the calculated urea concentration exceeds 50 mg/dL, dilute sample in distilled water and repeat the assay, multiplying by n.

Notes

- This assay is pH dependent; equilibrate all components to room temperature before each assay for consistent data.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.