BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Urea Assay Kit (Cat #: A-130)

COMPONENTS: UR Reagent 1: 5 ml (200 wells), store at room temperature (**contains H_2SO_4; handle with caution**)
UR Reagent 2: 0.1 ml, store at room temperature
UR Buffer: 20 ml, store at room temperature
Primaquine: 20 mg, store at room temperature
Urea: 100 mg, store at room temperature

PRODUCT DESCRIPTION: The Urea Assay Kit is based on a modified method (Biochem Cell Biol. 87:541–544, 2009) for measurement of urea concentration by light absorbance at 430-450 nm. Kit components are stable for at least one year if stored and handled properly.

PROTOCOLS

Urea Standards: Prepare a fresh 100 mM urea solution by dissolving 6 mg urea in 1 ml dH_2O. Dilute 100 mM urea 100x with dH_2O to 1 mM, e.g., 1 µl 100 mM urea + 99 µl dH_2O. Perform 1:1 serial dilution to obtain 500, 250, and 125 µM urea standards. Discard urea solution after each assay.

Primaquine solution- Prepare a fresh Primaquine solution by dissolving 1 mg Primaquine in 1 ml UR Buffer (1 mg/ml). Discard solution after each assay.

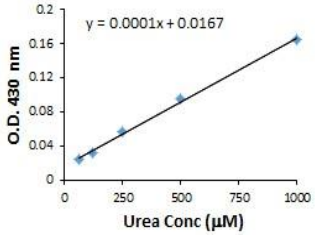
Working solution: Each urea/sample well requires 50 µl working solution consisting of 25 µl UR Reagent 1, 25 µl fresh Primaquine solution, and 0.5 µl UR Reagent 2. Calculate the amount of working solution required for each assay, including dH_2O blank, urea standards, and samples. Make enough working solution fresh prior to each assay.

Assay for cell culture medium and urine

1. Pipet 25 µl dH_2O or a suitable medium blank, urea standards, and samples to a plain (uncoated) 96-well plate.
2. Add 50 µl working solution to each well. Agitate plate for 10 sec to mix. Cover plate and incubate in a humidified 37°C incubator for 1 hour. *Do not use CO_2 incubator.*
3. Read absorbance (O.D.) at 430-450 nm using a plate reader. Subtract H_2O/blank well reading from urea standard and sample well readings. Use the subtracted urea standard readings to generate a urea standard plot and a trendline equation.
4. Sample urea concentration is derived from the trend line equation established at the same time (see graph below; x= urea concentration in µM; y= O.D.).

Assay for serum/plasma sample

1. Pipet 25 µl dH_2O (blank) and urea standards to a plain (uncoated) 96-well plate.
2. Pipet 25 µl serum/plasma sample to a set of 0.5-ml microtubes.
3. Add 50 µl working solution to the blank well and each urea standard well. Add 50 µl working solution to each serum/plasma tube followed by vortexing. Cover plate and close tubes. Incubate in a humidified 37°C incubator for 1 hour. *Do not use CO_2 incubator.*
4. Centrifuge serum/plasma tubes in a microfuge at ~14,000 rpm for 5 min. Transfer clear supernatant from each tube to the 96-well plate. *Avoid air bubbles and debris in wells.*
5. Follow steps 3-4 above for calculation of sample urea concentration.



NOTE:

- UR Reagent 1 contains sulfuric acid. Exercise caution and avoid skin contact.
- Please visit the product webpage or contact us for SDS information on sulfuric acid, primaquine, and phthaldialdehyde.