BIOMEDICAL RESEARCH SERVICE CENTER

UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Urea Assay Kit (Cat #: A-130)

COMPONENTS:
Urea Assay Solution-I: 5 ml, store at 4°C (for 200 wells; handle solution with caution)
Urea Assay Solution-II: 5 ml, store at 4°C
Urea Assay Solution-III: 0.15 ml, store at 4°C
Urea Standard: 1 g, store at room temperature

PRODUCT DESCRIPTION: The Urea Assay Kit is non-enzymatic, and is based on the modified Jung method (Biochem Cell Biol. 87:541–544, 2009), which involves a specific reaction of phthalaldehyde with urea, enabling measurement of urea by absorbance at 430 nm. Note that Urea Assay Solution-I contains 2.5M sulfuric acid, and should be handled with caution.

PROTOCOL

Working Assay Solution: Each well requires 50 µl freshly prepared Working Assay Solution, which consists of 25 µl Urea Assay Solution-I, 25 µl Urea Assay Solution-II, and 0.5 µl Urea Assay Solution-III. Calculate the amount of Working Assay Solution needed for standards and samples for each assay, and make enough Working Assay Solution in a 1.5-ml microtube or 4-ml culture tube prior to assay.

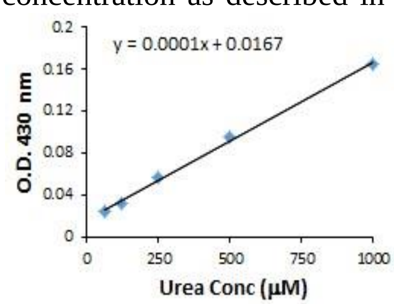
Urea Standard: Prepare fresh a 100 mM urea solution by dissolving 6 mg urea in 1 ml dH_2O. First dilute the 100 mM urea with dH_2O to 1 mM (1000 µM), i.e., 1 µl 100 mM urea + 99 µl dH_2O. Perform additional dilutions to obtain 500, 250, 125, and 62.5 µM standards. Discard urea solution after assay.

Assay for urine or cell culture medium sample (serum free)

1. Pipet 25 µl dH_2O (as urea standard blank) or plain culture medium (as medium blank), urea standards, and samples to each well of a 96-well plate.
2. Add 50 µl Freshly mixed Working Assay Solution to each well. Agitate plate gently for 10 sec. Incubate at room temperature in dark for 1 hour.
3. Read absorbance at 430 nm using a plate reader. Subtract the respective blank well reading from all urea and sample well readings. Use subtracted readings to generate the standard plot, and calculate sample urea concentration (see below).
4. Sample urea concentration is derived from the trend line of the urea standard plot established at the same time (see graph below; x= urea concentration in µM, y= $O.D._{430\ nm}$).

Assay for serum- or plasma-containing sample

1. Pipet 25 µl plain culture medium and samples to a set of 0.5-ml microtubes. Pipet 25 µl dH_2O and urea standards to each well of a 96-well plate.
2. Add 50 µl freshly mixed Working Assay Solution to each tube followed by brief vortexing. Add 50 µl Working Assay Solution to each well followed by gentle agitation for 10 sec. Incubate at room temperature in dark for 1 hour.
3. Centrifuge sample tubes in a microfuge at maximum speed (~13,000 rpm) for 5 min.
4. Transfer supernatant from each tube to a 96-well plate. Read absorbance at 430 nm using a plate reader.
5. Subtract the respective blank well reading from all sample and urea well readings. Use subtracted readings to generate a urea standard plot, and calculate sample urea concentration as described in steps 3 − 4 above.



NOTE:

- The assay format can be proportionately scaled up for cuvette measurement.
- Urea Assay Solution-I contains 2.5M sulfuric acid, which is corrosive. Handle with caution and avoid skin contact. Please visit the product webpage or contact us for MSDS information on sulfuric acid, phthalaldehyde, and primaquine bisphosphate.