BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Creatinine Assay Kit (Cat #: A-123)

COMPONENTS: CR Reagent 1: 20 ml (125 wells), store at room temperature (RT)
CR Reagent 2: 5 ml, store at RT
Creatinine: 100 mg, store at RT

PRODUCT DESCRIPTION: The Creatinine Assay Kit is based on Jaffe reaction, which is a chromogenic method used in clinical chemistry to measure serum/plasma and urine creatinine. The assay can be performed using a 96-well plate and measurement is based on light absorbance (O.D.) at 490-510 nm. The measurement can be done using the kinetic mode or endpoint mode. The kit is stable for at least one year if stored and handled properly.

Preparation of creatinine standard:

First prepare a 5 mM creatinine solution by dissolving 17 mg creatinine in 30 ml dH_2O.

Dilute a small aliquot of the 5 mM creatinine solution to 1 mM (1000 μM) using dH_2O, e.g., 80 μl dH_2O + 20 μl 5 mM creatinine.

Perform 1:1 serial dilution with dH_2O to obtain 500 μM, 250 μM, and 125 μM creatinine. Store the creatinine standard set at -20°C (good for ~3 months).

Preparation of fresh working solution:

Estimate the amount of working solution required for each assay and prepare the solution immediately prior to assay. Each creatinine standard/sample requires 0.2 ml working solution, which is prepared by mixing 4 parts of CR Reagent 1 and one part of CR Reagent 2.

Assay Protocol:

1. Use a plain (uncoated) 96-well plate for the assay. Add 10 μl of dH_2O, creatinine standards (125 - 1000 μM), and each sample to a well.

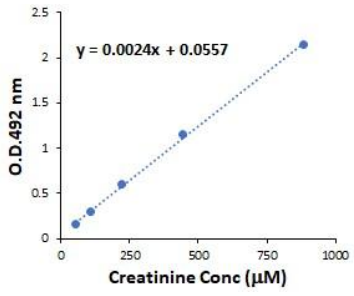
2. Add 0.2 ml working solution to each well. Gently agitate plate to mix. Allow reaction to proceed at room temperature for 10 min.

3. Read absorbance (O.D.) at 490-510 nm using a plate reader.

4. Subtract the dH_2O well reading from each creatinine standard well and each sample well reading.

5. Use the subtracted creatinine standard readings to generate a standard plot (see graph below; x= creatinine concentration in μM, y= $O.D._{490\text{-}510\ nm}$).

6. Use the subtracted sample readings for calculation of sample creatinine concentration as derived from the trend line equation of the standard plot established at the same time.



ADDITIONAL INFORMATION:

- Please visit the product webpage or contact us for material SDS information on NaOH and picric acid.