Ver.A261101

# Creatinine Assay Kit

ORG287 (100Tests)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

Creatinine is formed in muscles from phospho creatinine. It is an important form of energy, being a store of high-energy phosphate. Creatinine determinations have one advantage over Urea determination that it is not affected by a high protein diet. Serum creatinine is more specific & sensitive indicator of renal function. Simultaneous estimations of serum urea & creatinine provides better information. Serum urea nitrogen, creatinine ratio is 15 in pre renal failure, & < 10 in renal failure. Decreased levels are found in muscle dystrophy.

## Principle

Creatinine reacts with picric acid to produce a colored compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration

## Kit components

| Reagent | Volume | Description |
|---|---|---|
| Extraction Reagent | 110mL | 2-8°C |
| Creatinine Base Reagent (R1) | 2 × 50mL | 2-8°C |
| Creatinine Dye Reagent (R2) | 2 × 50mL | 2-8°C |
| Creatinine Standard (concentration 2mg/dL) | 4mL | 2-8°C |

## Open Vial Stability

Once opened, the reagent is stable up to 4 weeks at 2-8°C if contamination is avoided

## Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat bottom plate and distilled water.

## Reagent Deterioration

Turbidity or precipitation in any kit component indicates deterioration and vine component must be discarded. Values outside the recommended acceptable range for the Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested, using a fresh vial of reagent.

## Reagent Preparation

Mix one volume of Reagent 1 (R1) with one volume of Reagent 2 (R2). Creatinine standard is ready to use.

## Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

# Operation Procedures

## Sample Preparation

1. **Bacteria or cells**
   Harvest the cells and wash twice with PBS. Ideal to use 5 million cells for the assay. Add 1mL Extraction Reagent to 5 million cells and ultrasonicate (200W, work time 3 second / interval 10 second repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

   Note: Ideal proportion of Cells/Bacteria to Extraction Reagent is 1:5-10.
2. **Tissue**
   Prepare 10% tissue homogenate by adding 1mL Extraction Reagent to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant.
3. **Serum or Plasma**
   Directly use for the assay.

## Unit Conversion

| Traditional Unit | SI Unit | Conversion from Traditional to SI |
|---|---|---|
| mg/dL | µmol/L | × 88.4 |

## Procedure Notes

| | Standard | Sample |
|---|---|---|
| Working Reagent | 1000µL | 1000µL |
| Standard | 100µL | - |
| Serum | - | 100µL |
| Mix and read the optical density (T1) 60 seconds after the sample or standard addition. Take second reading (T2) exactly 60 seconds after the first reading | | |

## Calculation

$$\text{Creatinine conc. (mg/dL)} = \frac{(T1 - T2) \text{ of Sample}}{(T1 - T2) \text{ of Standard}} \times 2$$

# Performance

## Linearity

This reagent is linear up to 24mg/dL.
If the concentration is greater than linearity (24mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

## Sensitivity

Lower detection Limit is 0.13mg/dL.