**ELK Biotechnology**
**For research use only.**

# Creatinine Assay Kit Assay Kit Instruction (Cat/No. BC020)

## I. Reagent Compositions

| Reagent Name | Format:96T | Preservation Condition |
|---|---|---|
| Reagent I | 18mL | 4°C, avoid light |
| Reagent II | 6mL | 4°C, avoid light |
| Reagent III | 100μL | 4°C |
| 96-well plate | 1 plate | RT |

Assay kit should be kept at 2-8°C with 1 year validity.

## II. Principles of the Kit

$$Creatinine + H_2O + \xrightarrow{Creatininase} Creatine$$

$$Creatine + H_2O \xrightarrow{Creatinase} \text{Sarcosine} + \text{Urea}$$

$$Sarcosine + H_2O + O_2 \xrightarrow{Creatinase} \text{Glycine} + H_2O_2 + HCHO$$

$$H_2O_2 + 4 - AAP + ESPMT \xrightarrow{POD} Dyes + H_2O$$

Note: 4-AAP represents 4-aminoantipyrene and ESPMT represents N-ethyl-N-(3-sulfopropyl)-3-methyl aniline.

Dyes generated absorb light at 546 nm and the optical density (OD) is propotional to the concentration of creatinine. Record the OD at 546 nm to measure the concentration of creatinine.

## III. Procedures

| Reagents (μL) | Blank | Standard | Sample |
|---|---|---|---|
| Distilled Water | 6 | | |
| Reagent III | | 6 | |
| Sample | | | 6 |
| Reagent I | 180 | 180 | 180 |
| Incubate at 37°C for 5 min. Record the OD values $A_0$ at 546 nm. | | | |
| Reagent II | 60 | 60 | 60 |
| Incubate at 37°C for 5 min. Record the OD values $A_1$ at 546 nm.<br>Calculation $\Delta A=A_1- K\times A_0$. | | | |

Note: K is the dilution factor and

$$K = \frac{V_{Sample} + V_{Reagent\ I}}{V_{Sample} + V_{Reagent\ I} + V_{Reagent\ II}} = \frac{186}{246}$$

## IV. Calculation Formula and Reference Value

$$\underset{\mu M}{C_{Creatinine}} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times \underset{442\mu M}{C_{Standard}}$$

## V. Significance of the Kit

It's put in the muscle cell by metabolism last product of creatine, and creatine is dehydrated like non-enzyme, and you always do certain quantity metabolism of a creatinine (Cr). Non-protein in the blood, it's discharged in the urine almost all Cr's where nitrogenous subatance is one being filtered from renal glomerulus and without being reabsorbed. Therefore measurement of Cr in the blood will be an index of the filtration function by the kidney, and in addition to estimating the kidney function,

## VI. Notes

1. Urine samples should be diluted by saline with proper ratio before the measurement.
2. Sodium azide is added to reagent II and thus the reagent should be washed immediately if split on skin.
3. This kit is designed for research purpose only and cannot be used for diagnosis.
4. Different batches of kits are not recommended to be used simultaneously.