

## Intended Use

The reagents are used for the quantitative determination of Creatinine in serum or plasma. For in-vitro diagnostic use only.

## Introduction

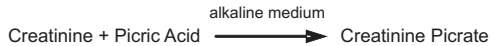
Creatinine is a break down product of creatine phosphate in muscles, and is usually produced at a fairly constant rate by the body. Chemically, creatinine is a spontaneously formed cyclic derivative of creatine. Approx. 2% of the body's creatine is converted into creatinine every day. Creatinine is transported through the blood stream to the kidney. There is little to no tubular re-absorption of creatinine by the kidney tubules. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). The GFR is important because it is a measurement of renal function. Any condition that impairs the function of the kidney will probably raise the creatinine level in the blood. In adults higher creatinine level is observed in high blood pressure, diabetes mellitus and intake of certain drugs.

## Method

Jaffe's method.

## Principle

Creatinine reacts with picric acid in alkaline medium forming a yellow orange color complex which is measured at 492nm:



The reaction is carried out up to fixed time points in order to minimize interference of other substances reacting with Picric acid.

## Reagent Composition

### Reagent 1:

|             |          |
|-------------|----------|
| Picric Acid | 9 mmol/l |
|-------------|----------|

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### Reagent 2:

|                    |             |
|--------------------|-------------|
| Sodium Hydroxide   | 400 mmol/l  |
| EDTA Disodium salt | 12.5 mmol/l |

### Reagent 3:

|                     |         |
|---------------------|---------|
| Creatinine Standard | 2 mg/dl |
|---------------------|---------|

### Precautions

- Following precautions should be taken:
- Avoid ingestion, do not pipette by mouth.
  - Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
  - Flush with plenty of water while disposing.

### Reagent Storage and Stability

Unopened Reagents are stable till expiry mentioned on the label when stored at 2-8°C.

Standard Reagent 3 is stable till expiry mentioned on the label when stored at 2-8°C.

**Note:** On request, Reagent 4 (Creatinine: 10mg/dl) & Reagent 5 (Creatinine: 20mg/dl) can be provided for linearity check with Reagent 3 (Creatinine 2mg/dl - Standard).

### Reagent Preparation

Preparation of working reagent

Mix 1 volume of Reagent R1 with 1 volume of Reagent R2. Working solution is stable for seven days if stored at 2-8°C.

### Reagent Deterioration

Reagent should be clear. Turbidity and/or precipitation may be because of reagent deterioration.

### Sample Collection and Storage

Serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. For urine samples, dilute the urine sample 1/50 with distilled water.

Samples can be stored for 7 days at 2-8°C and 24hr at room temperature.

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## General Assay Parameters

| Mode                       | Fixed Time |
|----------------------------|------------|
| Wavelength 1 (nm)          | 492        |
| Wavelength 2 (nm)          | -          |
| Blank with                 | Air        |
| Sample Volume (µl)         | 50/100     |
| Working Reagent (µl)       | 500/1000   |
| Delay Time (sec)           | 30         |
| Read Time (sec)            | 90         |
| No. of readings            | 1          |
| Incubation Temperature(°C) | 37         |
| Normal Low (mg/dl)         | 0.5        |
| Normal High (mg/dl)        | 1.5        |
| Linearity (mg/dl)          | Upto 20    |
| Standard Conc. (mg/dl)     | 2          |
| Units                      | mg/dl      |

## PROCEDURE

Pre-warm working reagent at 37°C for 2 minutes prior to addition of sample.

### Pipette into test tubes:

|                 | Standard | Sample |
|-----------------|----------|--------|
| Working Reagent | 1000µl   | 1000µl |
| Reagent 3       | 100µl    | -      |
| Sample          | -        | 100µl  |

Mix well & read initial absorbance A1 exactly after 30 sec of mixing. Read final absorbance A2 after 90 sec. at 37°C.

## Calculation

Creatinine concentration in the sample can be calculated using the following formula:

$$\Delta A = A2 - A1$$

where,  $\Delta A$  = change in absorbance

$\Delta \text{Abs of Sample}$  = change in the absorbance of sample

$\Delta \text{Abs of Standard}$  = change in the absorbance of standard

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**Example:** If the  $\Delta$  Absorbance of sample is 0.200 and the  $\Delta$  Absorbance of standard is 0.18. The calculation shall be:

$$\text{Creatinine} = \frac{\Delta \text{Abs of Sample}}{\Delta \text{Abs of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

If the creatinine concentration exceeds 20mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

$$\frac{0.200}{0.180} \times 2.0 = 2.22 \text{ mg/dl}$$

## Reference value

Serum

Male : 0.6 – 1.5mg/dl

Female : 0.5 – 1.3mg/dl

Reference range varies from population to population; therefore, each laboratory should establish its own normal range.

## Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample:reagent ratio remains same.
2. Temperature has to be maintained constant throughout the reaction as the rate of color development is highly temperature sensitive.

## Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for creatinine.

## Performance

**Linearity Limit:** 20mg/dl

**Precision:**

**Within run**

| Control        | Control 1 | Control 2 |
|----------------|-----------|-----------|
| No. of samples | 20        | 20        |
| Mean (mg/dl)   | 1.32      | 7.17      |
| S.D.           | 0.02      | 0.10      |
| C.V. %         | 1.74      | 1.34      |

**Between run**

| Control        | Control 1 | Control 2 |
|----------------|-----------|-----------|
| No. of samples | 60        | 60        |
| Mean (mg/dl)   | 1.32      | 7.18      |
| S.D.           | 0.02      | 0.10      |
| C.V. %         | 1.70      | 1.35      |

## References

1. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz (2005). "Diabetic nephropathy: diagnosis, prevention, and treatment". Diabetes Care 28 (1): 164–76. doi:10.2337/1diacare.28.1.164. PMID 15616252.
2. Delanghe J; De Slypere JP, De Buyzere M, Robbrecht J, Wieme R, Vermeulen A (Aug 1989). "Normal reference values for creatine, creatinine, and carnitine are lower vegetarians" (PDF). Clin. Chem. 35 (8): 1802–3. PMID 2758659
3. Lamb, E; Newman, D. J. & Price, C. P. 'Kidney Function Tests' in Tietz Textbook of clinical chemistry and Molecular Diagnostics. Burtis, C. A., Ashwood, E. R. & Bruns, D. E. (Eds). Elsevier Inc., Fourth Edition. 2006; 24:797-835.

## Pack Presentation

| Product Code/<br>Catalogue No. | KGCRE<br>101.6.1 | KGCRE<br>101.6.2 | KGCRE<br>101.6.3 |
|--------------------------------|------------------|------------------|------------------|
| Pack Size *                    | 2X50ml           | 4X50ml           | 4X100ml          |
| Reagent 1                      | 1x50ml           | 2X50ml           | 2X100ml          |
| Reagent 2                      | 1X50ml           | 2X50ml           | 2X100ml          |
| Reagent 3                      | 1X2ml            | 1X3ml            | 1X3 ml           |

\*Pack size may vary on market demand.

Revision No: (Ver: KGCRE101.6/1)

Date of Issue: 1st April 2010

## Symbols

Following symbols are used in the labeling of KEE GAD kits:



Catalogue No.



Batch No.



CE Mark



Read instructions



In Vitro Diagnostics



Storage temperature



Expiry Date



Content



Product Name



Manufactured By



**Manufactured by:**  
**KEE GAD Biogen Pvt. Ltd.**  
 A-8, Third Floor, Naraina Industrial Area,  
 Phase-II, New Delhi-110028 (India)