**Creatinine Reagent**  
(Single Vial)  
Catalog #: 80136  
for use with the LIASYS-330 CLINICAL CHEMISTRY SYSTEM

**INTENDED USE**  
For the in vitro quantitative determination of Creatinine in serum.

**SUMMARY AND EXPLANATION**
Creatinine measurements are used in the diagnosis and treatment of renal function, including diseases, monitoring renal dialysis, and as a calculation basis for measuring other urine analytes. Elevated Creatinine levels are found in renal diseases and insufficiency with decreased glomerular filtration (uremia or azotemia if severe); urinary tract obstruction; reduced renal blood flow including congestive heart failure, shock and dehydration; rhabdomyolysis causes high serum creatinine, which may be elevated out of proportion to BUN, or to the reduction in renal function.

**METHODOLOGY**
Jaffe\(^1\) described a method in 1886 for the determination of creatinine involving a protein free filtrate and a reaction with picric acid in alkaline solution. Although since then several methods have been described the classic Jaffe reaction method is still the most widely used. The Jaffe reaction is subject to interferences by a number of substances, including protein and glucose.\(^2\)\(^3\)\(^4\)\(^5\) Modifications of the procedure have been developed to combat the drawbacks.\(^6\) The kinetic procedures\(^7\) have become popular because they are fast, simple and avoid interferences. This method is based on a modification of the above procedure, incorporating a surfactant and other ingredients to minimize protein and carbohydrate interferences.

**PRINCIPLE**
Creatinine reacts with picric acid in alkaline conditions to form a color complex which absorbs at 510 nm. The rate of formation of color is proportional to the creatinine in the sample.

**REAGENT COMPOSITION**

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Picric Acid</td>
<td>10 mM</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>250 mM</td>
</tr>
<tr>
<td>pH 13.0 ± 0.2</td>
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</tbody>
</table>

**PRECAUTIONS**
1. This reagent is for in vitro diagnostic use only.
2. Picric Acid is a strong oxidizing agent. Avoid contact with skin. WIPE ANY SPILLAGE, SINCE EVAPORATED PICRIC ACID IS EXPLOSIVE.
3. Sodium hydroxide is an alkali. Avoid ingestion and contact.

**REAGENT PREPARATION**
Reagent is supplied as a single vial ready to use liquid.

**REAGENT STORAGE**
1. Store the reagent at 2-8°C (refrigerated).
2. The reagent is stable until the expiration date when stored at 2-8°C.
3. Reagent should be protected from light when not in use.

**REAGENT DETERIORATION**
The reagent should not be used if:
1. The reagent is cloudy (contaminated).
2. The reagent fails to meet stated parameters of performance.
3. The initial reagent absorbance is greater than 0.330 at 500 -510nm.

**SPECIMEN COLLECTION AND STORAGE**
1. Un-hemolyzed serum is recommended.
2. Creatinine in serum is stable for 7 days at refrigerated temperatures (2-8°C) and for several months when frozen (-20°C) and protected from evaporation and contamination.

**INTERFERENCES**
Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

- **Hemoglobin**: No significant interference (±10%) from hemoglobin up to 400 mg/dL.
- **Bilirubin**: No significant interference (±10%) from bilirubin up to 5.6 mg/dL.
- **Lipemia**: No significant interference (±10%) from lipemia up to 420.5 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of creatinine. See Young, et al.\(^9\)

**ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED**
1. LIASYS 330 Clinical Chemistry System
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Controls and Calibrator materials such as those provided by AMS Diagnostics.

**ASSAY PROCEDURE**
These instructions are to be used as a general guideline for adapting to select automated instruments. Refer to your specific instrument application instructions available upon request.

**SYSTEM PARAMETERS**
- **Temperature**: 37°C
- **Wavelength**: 510 nm
- **Assay Type**: Fixed Rate
- **Direction**: Increase
- **Sample/Rgt. Ratio**: 1: 10
  - e.g. Sample Vol. 0.1 mL (100mL)
  - Reagent Vol. 1.0 mL
- **First Read Time**: 60 Sec
- **Delay Time**: 120 Sec
- **Last Read Time**: 180 Sec

**PROCEDURE NOTES**
The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

**CALCULATIONS**

\[
\text{Creatinine (mg/dL)} = \frac{\text{Absorbance}}{\text{Sensitivity}} \times \text{Concentration of standard}
\]

**EXAMPLE**

\[
\text{Example:} \quad \text{A (standard)} = 0.01
\]

**LIMITATIONS**
Samples with values exceeding linearity should be diluted 1:1 with saline and re-run. The final answer should be multiplied by two.

**QUALITY CONTROL**
Use an aqueous Creatinine standard or an appropriate serum calibrator.

**EXPECTED VALUE**
Serum: 0.60 – 1.40 mg/dL

**REFERENCES**

It is highly recommended that each laboratory establish its own reference range.

**PERFORMANCE**
- **Linearity**: When run as recommended the assay is linear from 0.1 to 20 mg/dL

**Method Comparison**
Studies performed between this procedure and a similar methodology yielded the following results:

- **Number of samples pairs**: 57
- **Range of samples**: 0.4 – 23.4 (mg/dL)
- **Correlation Coefficient**: 0.9989
- **Slope**: 0.9576
- **Intercept**: -0.07 (mg/dL)

**SENSITIVITY / LIMIT OF DETECTION**
A calibration factor of approximately 63.127 was obtained, which is equivalent to a sensitivity of 0.016 µg/L.

The lower Limit of Detection was found to be 0.1 mg/dL.

**PI Rev 02/26/09**