INTRODUCTION

1. AutoZyme Creatinine is a reagent set for determination of creatinine based on **initial rate method** using Alkaline Picrate.
2. AutoZyme Creatinine is a **single reagent system,** using one step procedure.
3. AutoZyme Creatinine has **one step reconstitution.** It involves mixing of Picrate and Diluent reagent.
4. AutoZyme Creatinine is a **High Stability Reagent.**
5. AutoZyme Creatinine is **linear** upto 30 mg%.
6. Creatinine can be determined in **180 seconds.**
7. AutoZyme Creatinine can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.

PRINCIPLE

Creatinine in alkaline medium reacts with picrate to produce orange colour. This colour absorbs light at 492 nm.(490 - 510 nm.). The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen.

Creatinine + Picrate —(Alkaline medium)→ **Orange colour**

PREPARATION OF WORKING SOLUTION

Prepare working solution by mixing **equal volume of Picrate Reagent** and **Diluent Reagent.**

REAGENT STORAGE & STABILITY

The reagents are stable till the expiry date stated on the bottle label, when stored at R.T. (25-30°C).

The working solution is stable for 30 days at 2-8°C.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Sodium Picrate	7.7 mmol/l
• Sodium Hydroxide	500 mmol/l

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Avoid use of plastic or siliconized container which may prolong clotting time. Samples should not be collected during PSP/BSP clearance test. For plasma separation Heparin (200 IU/ml blood) may be used as anticoagulant.

Creatinine in serum and plasma is stable for 2 days when stored at 2-8°C.

PROCEDURE

☐ Reaction type	Initial rate
☐ Reaction direction	Up
☐ Wavelength	492 nm.(490 - 510 nm.)
☐ Flowcell temperature	30°C / 37°C
☐ Zero setting with	Distilled water
☐ Delay time	30 seconds
☐ No. of readings	2
☐ Interval	60 seconds
☐ Sample volume	0.05 ml (50 µl)
☐ Reagent volume	1.0 ml
☐ Standard concentration	2 mg %
☐ Factor	2 ÷ Δ Abs. of standard
☐ Linearity	30 mg/dl

Manual assay procedure

Prewarm the required amount of working solution to 30°C/37°C before use.

1.0 ml procedure

Standard / Sample	0.05 ml (50 µl)
Working Solution	1.0 ml

Mix and start stopwatch simultaneously. Record absorbance assay mixture at exactly 30 seconds after Standard / Specimen addition and then again at 90 seconds.

Note : It is recommended to run a creatinine standard with each batch of assay.

Calculation:

Calculate the average change in absorbance per minute (Δ Abs.) of standard & specimen(s).

Δ Abs. = Abs. at 90 sec. — Abs. at 30 sec.

$$\text{Serum Creatinine (mg\%)} = \frac{\Delta \text{ Abs. of Specimen}}{\Delta \text{ Abs. of Standard}} \times 2$$

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DETERMINATION OF URINE CREATININE

Sample Collection

Creatinine determination in urine is usually carried out on a 24 hour urine sample. Thymol as preservative should be used for collection. The urine specimen should be thoroughly mixed and then diluted 1 : 25 with distilled water.

Urine samples containing thymol as preservative are stable for one week at 2-8^{0}C.

PROCEDURE

Follow the same procedure as given before.

Calculation :

$$\text{Urine Creatinine (mg\%)} = \frac{\Delta \text{ Abs. of Specimen}}{\Delta \text{ Abs. of Standard}} \times 2 \times 25$$

EXPECTED VALUES

SERUM CREATININE

MALE : 0.7 - 1.2 mg%

FEMALE : 0.5 - 1.0 mg%

URINE CREATININE

MALE : 21-26 mg/kg. body weight / 24 hrs.

FEMALE : 16-22 mg/kg. body weight / 24 hrs.

PROCEDURE LIMITATIONS

1. If the creatinine value exceeds 30 mg% dilute the specimen with equal volume of distilled water and reassay. Multiply the results by two to obtain correct creatinine value.
2. Discard the working solution if the adsorbance is more than 0.200 against distlled water at 492 nm.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware, and accuracy of pipetting.

REFERENCES

1. Henry,R.J., et al. ***"Clinical Chemistry - Principles and Techniques"*** Harper & Row, II Ed (1974).
2. Larson K., ***Clin. Chem Acta. 41,*** 209, (1972).

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE European Conformity

AR. No.: I 11 *CR-2009-03-00I*

AutoZyme Creatinine

AutoZyme

CREATININE

Initial Rate

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