ANA BIO CLR Creatinine

(Jaffe (initial rate) method using Alkaline picrate)

Intended Use

Creatinine is a reagent kit used for the determination of Creatinine in serum/plasma or in urine based on initial rate method using alkaline picrate.

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 $\xrightarrow{\text{Alkaline medium}}$ Creatininepicrat (Orange Colour)

Reagents provided

1. R1 - Picrate reagent
2. R2 - Diluent reagent
3. Creatinine standard (2 mg/dl)

Working Reagent Preparation

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

Reagent storage and stability

The reagents are stable till the expiry date stated on the bottle label, when stored at room temperature (25 - 30℃). The working solution is stable for 30 days at 2° - 8℃.

DETERMINATION OF SERUM/PLASMA CREATININE

Specimen collection and preservation

Blood should be collected in a clean and 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 of blood) may be used as anticoagulant. Creatinine in serum and plasma is stable for 2 days when stored at 2° - 8℃.

Assay guidelines for Analyzers

Reaction type	Fixed time (Two Points)
Reaction Slope	Increasing
Wavelength	492 nm (490 - 510 nm)
Temperature	37℃
Delay time	30 seconds
Interval time	90 Seconds
Blank	Distilled water
Sample volume	100 µl (0.1 ml)
Reagent volume	1000 µl (1.0 ml)
Creatinine Standard Concentration	2 mg/dl
Factor Calculation	2 mg/dl ÷ Absorbance of Standard
Low Normal	0.7 mg/dl
High Normal	1.2 mg/dl
Linearity	Up to 30 mg/dl

Assay guidelines for Manual Procedure

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

Reagents	**Standard**	**Test**
Working Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	100 µl (0.1 ml)	-
Sample	-	100 µl (0.1 ml)

1. Mix the above assay mixture thoroughly.
2. Read and record absorbance exactly at 30th second (A1) at 492 nm (490 - 510 nm) and then again read the absorbance exactly at 120th second (A2).
3. Calculate change in absorbance Δ A by subtracting A2 – A1.

Calculation

$$\text{Serum / Plasma Creatinine (mg/dl)} = \frac{\text{Sample OD.}}{\text{Std. OD.}} \times \text{Con. of Std.}$$

DETERMINATION OF URINE CREATININE

Specimen collection

Creatinine determination in urine is usually carried out on 24 hrs. 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°C.

Procedure

Follow the same procedure as given before.

Calculation for urine sample

Urine Creatinine (mg/dl) = $\frac{\text{Sample OD.}}{\text{Std. OD.}}$ x 2 x 25

Normal Range

Guidance Value for Male	Serum	0.7 - 1.2 mg/dl
	Urine	21 - 26 mg/kg. Body weight / 24 hrs.
Guidance Value for Female	Serum	0.5 - 1.0 mg/dl
	Urine	16 - 22 mg/kg. Body weight / 24 hrs.

Note: Expected range varies from population to population and each laboratory should establish their own normal range.

Limitation

1. Reaction is linear up to 30 mg/dl. For higher values, dilute the sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.
2. Discard the working solution if the absorbance is more than 0.200 against distilled water at 492 nm.

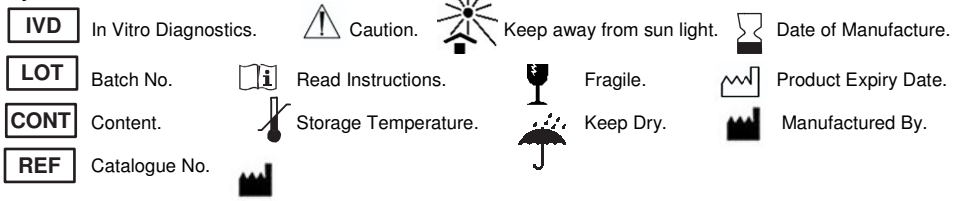
Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized 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, and cleanliness of glassware and accuracy of pipetting.

References

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

Symbols



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