CUSTOMBIOTECH

Creatinine

Test principle: Enzymatic colorimetric

creatinine + H_2O $\xrightarrow{\text{creatininase}}$ creatine

creatine + H_2O $\xrightarrow{\text{creatinase}}$ sarcosine + urea

sarcosine + O_2 + H_2O $\xrightarrow{\text{sarcosine oxidase}}$ glycine + formaldehyde + H_2O_2

H_2O_2 + 4-aminoantipyrine/HTIB $\xrightarrow{\text{POD}}$ H_2O + dye

removal of ascorbate: 2 L–ascorbate + O_2 + H_2O $\xrightarrow{\text{ascorbate oxidase}}$ 2 L-dehydroascorbic acid + 2 H_2O

Creatinine is produced endogenously from creatine and creatine phosphate as a result of muscle metabolic processes. It is excreted by glomerular filtration during normal renal function. Creatinine assays are conducted for diagnostic purposes, for therapeutic monitoring of acute and chronic renal diseases, and for monitoring kidney dialysis. The urinary creatinine concentration can also be used as a reference parameter for analyte excretion (albumin, a-amylase). Numerous methods have been described for determining creatinine, including the Jaffé alkaline picrate method in various modifications, as well as an enzymatic test which involves measuring ammonia after cleavage of creatinine by creatinine iminohydrolase.

The enzymatic method is based on the established determination of hydrogen peroxide after conversion of creatinine with the aid of creatininase (creatinine amidohydrolase), creatinase, and sarcosine oxidase. The liberated hydrogen peroxide reacts with 4-aminoantipyrine and HTIB to form a quinone imine chromogen. The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration and is measured photometrically.

Proposed reagent composition approximately 2+1 formulation

Reagent 1

Composition	Concentration	Catalog Number
Buffer (TAPS, pH 8.1)	30 mmol/l	11 120 425 001
Creatinase	>20 kU/l	11 799 142 103
Sarcosine oxidase	>8 kU/l	11 378 856 103
Ascorbate oxidase	>2 kU/l	11 558 668 103
Catalase	>0.1 kU/l	11 650 645 103
HTIB	5.9 mmol/l	
Detergent, preservative		

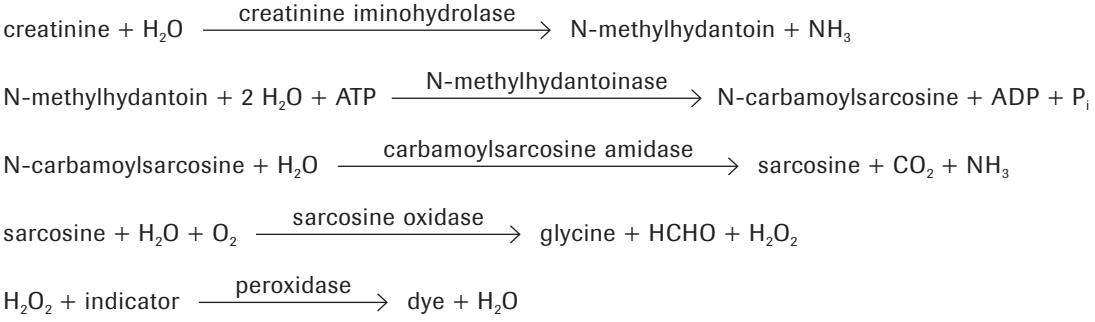
- Endogenous creatine in the sample is destroyed by creatinase, SOD, and catalase during incubation in R1.
- Avoid sodium azide in R1.

Reagent 2

Composition	Concentration	Catalog Number
Buffer (TAPS, pH 8.0)	50 mmol/l	11 120 425 001
Creatininase	>30 kU/l	11 865 471 103
Peroxidase	>1 kU/l	11 378 783 103
4-Aminoantipyrine	2 mmol/l	10 073 474 103
Potassium hexacyanoferrate (II)	0.163 mmol/l	
Detergent, such as Triton X-100	0.01 %	10 743 119 103
Preservative, such as Sodium azide		

Products are for further processing only.

The following method is suitable for dry chemistry systems:



Roche Enzymes for this assay

Products	Catalog Number
Creatinine Deaminase (Creatinine iminohydrolase)	11 330 764 103
N-Methylhydantoinase (ATP-hydrolyzing)	11 288 555 103
N-Carbamoylsarcosine Amidase	11 248 847 103
Sarcosine oxidase	11 378 856 103
Peroxidase	11 378 783 103

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