May 14, 2019

Working

UC Davis - Creatinine Protocol

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dx.doi.org/10.17504/protocols.io.ygjftun

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ABSTRACT

Summary:

The method employed here is based on an enzymatic colorimetric determination of creatinine which largely eliminates interferences known to the Jaffe method. In the final reaction sequence, the formation of the quinoneimine dye product results in an increase in absorbance at 550 nm (530-570 nm) which is directly proportional to the creatinine concentration in the sample. Potential interference from endogenous creatine and sarcosine are eliminated by the reaction of creatine amidinohydrolase, sarcosine oxidase and catalase before creatinine is determined. Ascorbate oxidase is included in the reagent to eliminate the influence of ascorbate in the sample.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=99&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Calibrator	TR43002	Fisher Diagnostics		
Reagents	TR35401	Fisher Diagnostics		
Microplate				
Platereader				

MATERIALS TEXT

Note:

Thermo Fisher Scientific, RRID:SCR_008452

1 Add 3 µl of calibrator and sample to each well.

2 Add 135 µl of R1 to each well. Incubate at 37°C for 5 minutes. Read at 550 nm.

IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

3 Add 45 µl of R2 to each well. Incubate at 37°C for 5 minutes. Read at 550 nm.

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4 Subtract blank readings from final readings. The assay will be linear so the unknown samples can be calculated as (Sample Absorbance ÷ Calibrator Absorbance) × Calibrator Concentration.

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