May 14, 2019

UC Davis - Creatinine Protocol

DOI
https://dx.doi.org/10.17504/protocols.io.ygjftun

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DOI: https://dx.doi.org/10.17504/protocols.io.ygjftun

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

Protocol Citation: Peter Havel 2019. UC Davis - Creatinine Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ygjftun

Protocol status: Working
We use this protocol and it's working

Created: February 23, 2019

Last Modified: May 14, 2019

Protocol Integer ID: 20715

Keywords: creatinine, enzymatic colorimetric determination of creatinine, creatinine concentration, creatinine protocol summary, creatine amidinohydrolase, potential interference from endogenous creatine, reaction of creatine amidinohydrolase, enzymatic colorimetric determination, catalase before creatinine, endogenous creatine, proportional to the creatinine concentration, quinoneimine dye product result, formation of the quinoneimine dye product result, ascorbate oxidase, sarcosine oxidase, oxidase, increase in absorbance, sarcosine

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.

Materials

MATERIALS

Calibrator **Fisher Diagnostics Catalog #**TR43002

Reagents **Fisher Diagnostics Catalog #**TR35401

Microplate

Platereader

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.

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.