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UC Davis - Creatinine Protocol

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OPEN ACCESS

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

Created: Feb 22, 2019

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PROTOCOL integer ID:
20715

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

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Calibrator Fisher Diagnostics Catalog #TR43002

Reagents Fisher Diagnostics Catalog #TR35401

Microplate Contributed by users

Platereader Contributed by users

Note:

Thermo Fisher Scientific, [RRID:SCR_008452](#)

Keywords: creatinine

- 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 $(\text{Sample Absorbance} \div \text{Calibrator Absorbance}) \times \text{Calibrator Concentration}$.