May 15, 2019

Working

UC Davis - Urea Protocol

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dx.doi.org/10.17504/protocols.io.yw6fxhe

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ABSTRACT

Summary:

The enzyme methodology employed in this reagent is based on the reaction first described by Talke and Schubert. To shorten and simplify the assay, the calculations are based on the discovery of Tiffany, et al. that urea concentration is proportional to absorbance change over a fixed time interval. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm as NADH is converted to NAD.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=100&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Calibrator	TR43002	Fisher Diagnostics
Reagents	TR12003	Fisher Diagnostics
Microplate		
Platereader		

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

2 Add 300 µl of reagent to each well. Incubate at 37°C for 30 seconds. Read at 340 nm.

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

3 Incubate at 37°C for 60 seconds. Read at 340 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.