May 14, 2019

UC Davis - Glucose Protocol

DOI
dx.doi.org/10.17504/protocols.io.yj9fur6

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.yj9fur6

External link: https://mmpc.org/shared/document.aspx?id=88&docType=Protocol

Protocol Citation: Peter Havel 2019. UC Davis - Glucose Protocol. **protocols.io https://dx.doi.org/10.17504/protocols.io.yj9fur6**

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

Created: February 26, 2019

Last Modified: May 14, 2019

Protocol Integer ID: 20833

Keywords: glucose, glucose oxidase, glucose oxidase to gluconic acid, glucose protocol summary, peroxidase with hba, peroxidase, glucose concentration, red quinoneimine dye, hydrogen peroxide reacts in the presence, hydrogen peroxide react, hydrogen peroxide, gluconic acid, proportional to the glucose concentration, oxidase, color, intensity of the color

Abstract

Summary:

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. The hydrogen peroxide reacts in the presence of peroxidase with HBA and 4-aminoantipyrine forming a red quinoneimine dye. The intensity of the color formed is proportional to the glucose concentration and can be measured photometrically between 460 and 560 nm.

Materials

MATERIALS

- Calibrator **Fisher Diagnostics Catalog #**TR1591-030
- Reagents **Fisher Diagnostics Catalog #**TR15103
- PBS
- Microplate
- Platereader

Reagent Preparation:

PBS – ready to use
Reagent – reconstitute with distilled water to make a 2X solution

Troubleshooting

1 Reconstitute powdered reagent with only 25 ml of distilled water to make a 2X solution.

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

3 Add 150 µl of PBS to each well. Read at 540 nm.

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

4 Add 150 µl of 2X reagent to each well. Incubate at 37°C for 10 minutes. Read at 540 nm.

5 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.