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UC Davis - Glucose Protocol

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

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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

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.