Glucose Protocol

Version: 1
Replaced by version
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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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Calibrator	Fisher Diagnostics	TR1591-030
Reagents	Fisher Diagnostics	TR15103
PBS		
Microplate		
Platereader		

Protocol:

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.

Reagent Preparation:

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