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Working

UC Davis - Glucose Protocol

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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.

EXTERNAL LINK

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

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Calibrator	TR1591-030	Fisher Diagnostics		
Reagents	TR15103	Fisher Diagnostics		
PBS				
Microplate				
Platereader				

MATERIALS TEXT

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.