

Protocol 4. The Nelson-Somogyi method for reducing sugars [7]

- Sensitivity: ~ 10-100 μg glucose in 1 ml
- Final volume: 6.0 ml^b (~ 60-600 μM)

Reagents

A Dissolve 15 g of sodium potassium tartrate and 30 g of anhydrous Na_2CO_3 in about 300 ml water. Add 20 g NaHCO_3 . Dissolve 180 g of anhydrous Na_2SO_4 in 500 ml boiling water and cool. Mix the two solutions and make up to 1 litre with water.^a

B Dissolve 5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 45 g anhydrous Na_2SO_4 in water and make up to 250 ml.^a

C. Mix reagents A (4 vol.) and B (1 vol.) just before use.

D Dissolve 25 g ammonium molybdate in 450 ml water. carefully add 21 ml concentrated H_2SO_4 with stirring. Dissolve 3 g $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ in 25 ml water and add to the molybdate solution. Incubate for 24-28 h at 37°C and store in a brown glass-stoppered bottle. ^a Just before use this reagent should be diluted with 2 vol. of 0.75 M H_2SO_4 (4 ml concentrated H_2SO_4 in 100 ml solution).

Method

1. Mix the samples, standards, and control solutions (1.0 ml) containing up to 800 nmol reducing sugar with 1.0 ml of reagent C in small stoppered test-tubes.
2. Heat at 100°C for 15 min. Cool the solution rapidly to room temperature.
3. Add reagent D (1.0 ml) and mix well.
4. Add 3.0 ml water, mix, and measure the absorbance at 520 nm.

^a The reagents are stable in their concentrated unmixed forms.

^b Care should be taken to minimize reoxidation by air (see Protocol 3).