Protocol carbohydrate determination

*According to the phenol-sulphuric acid or DuBois method (modified to fit laboratory materials)*

## Materials:

* 1 cm discardable PS cuvettes
* UV-VIS spectrophotometer
* 50 g/L phenol (0.6 mL/sample)
* 95-99% H2SO4 (3 mL/sample)
* Calibration curve: 0-50 mg/L glucose
* mQ water

## Methods:

*Preparation of calibration standards*

* Prepare a stock solution of 500 mg glucose/L **(you may keep this up to 2 weeks in the fridge)**
* In glass beakers/volumetric glassware, prepare the calibration standards:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Blank | 2.5 mg/L | 5 mg/L | 10 mg/L | 25 mg/L | 50 mg/L |
| BSA stock | 0 µL | 125 µL | 250 µL | 500 µL | 1250 µL | 2500 µL |
| mQ water | 25 mL | 24.875 mL | 24.75 mL | 24.5 mL | 23.75 mL | 22.5 mL |

*Protocol for measurement preparation (calibration and samples)*

* Switch on spectrophotometer.
* Add 1.5 mL of sample/calibration to **glass** test tube (solutions will easily reach 80°C, enough to soften most plastics).
* Add 0.6 mL of phenol solution.
* Add 3 mL of sulphuric acid – samples will become hot. DO NOT COOL – a hot acidic medium is required for the reaction to occur.
* Vortex the solutions for 30 s to ensure good mixing (sulphuric acid has the tendency not to mix very well).
* Incubate for 30 min at room temperature.
* **Measure your samples at 487 nm (samples should have an orange-red colour).**

## Results:

Prepare your own calibration curve! Normal conditions should produce a slope of about 0.00955 Abs/mg glucose/L (may differ for different carbohydrates).