Protocol protein determination

*According to the Lowry method (modified to fit laboratory materials)*

## Important notes:

* The calibration range cannot be extended further! Samples between the 0 and 25 mg/L standard are not to be quantified (you can only qualitatively conclude whether proteins are there or not).
* Samples containing whole cells or precipitates need to be treated differently (e.g. by using a hydrolysis pretreatment).
* This method is sensitive to many types of buffers, detergents, chelating agents, cyclic organic compounds and other types of reducing compounds.

## Materials:

* 1 cm discardable PS cuvettes
* UV-VIS spectrophotometer
* Reagent A: 143 mM NaOH, 271 mM Na2CO3 (27.45 µL/sample)
* Reagent B: 57 mM CuSO4 (27.45 µL/sample)
* Reagent C: 124 mM Na-K-tatrate, C4H4NaKO6 (2.745 mL/sample)
* Folin-Ciocalteu phenol reagent (15.2 in cupboard) (0.4 mL/sample)
* Calibration curve (see below for preparation): 25-500 mg/L BSA (Bovine Serum Albumin)
* mQ for all dilutions

## Methods:

*Preparation of calibration standards*

* Prepare a stock solution of 500 mg BSA/L **(you may keep this up to 2 weeks in the fridge)**
* In test tubes (glass or PS), prepare the calibration standards (rest of solutions will be added to this test tube):

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Blank | 25 mg/L | 50 mg/L | 100 mg/L | 250 mg/L | 500 mg/L |
| BSA stock | 0 µL | 100 µL | 200 µL | 400 µL | 1000 µL | 2000 µL |
| mQ water | 2000 µL | 1900 µL | 1800 µL | 1600 µL | 1000 µL | 0 µL |

*Protocol for measurement preparation (calibration and samples)*

* Prepare D solution: mix A, B and C in a volume ratio of 100:1:1.
* Switch on spectrophotometer.
* Add 2 mL of sample to test tube (for calibration standards, you already did this).
* Add 2.4 mL of D solution to test tube.
* Incubate for 10 min at room temperature.
* Add 0.4 mL of Folin-Ciocalteu phenol reagent to test tube.
* Incubate for 45 min at room temperature.
* **Measure your samples at 750 nm (samples should have a blue colour).**

## Results:

Prepare your own calibration curve! Normal conditions yield a second-order polynomial, and should produce B2, B1 and B0 values of -2.49. 10-6, 3.67.10-3 and 2.86.10-2 (may differ for different proteins and fit.).