Protein Quantification with Lowry Method at 750 nm Measurement

MATERIAL

- BSA (Sigma-Aldrich, St Louis, MO, U.S.A.) 1 mg/ml diluted in 0.01 M PBS pH 7.4.
- Lowry reagents: 0.1 M NaOH, 2 % Na_2CO_3 in 0.1 M NaOH (reagent A), 1 % Na-tartrate in ddH_20 (reagent B), 0.5 % CuSO4 in ddH_20 (reagent C), 1:1 Folin-Ciocalteu's phenol reagent in ddH_20 (Protein and chemicals all from Sigma-Aldrich)
- Standard curve with concentrations 1 µg/ml, 2 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml. Diluted in 0.1 M NaOH.
- SpectroArt 200, Protein Function, Lowry 750 nm, 7 standard curve points, read blank 2 times and samples 5 times.
- Hellma cuvettes: 5 µl TrayCell with 1 mm PL, 100 µl black wall cuvette 10 mm PL, 700 quartz cuvette, 10 mm PL. Plastic disposable cuvettes, 3 ml 10 mm PL.
- ddH_20 and 70 % ethanol for washing.
- Cotton buds for cleaning of TrayCell.

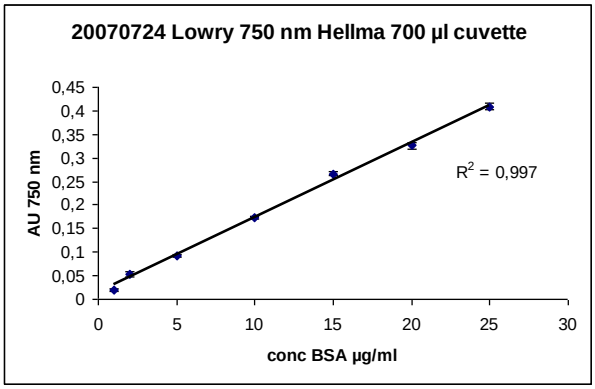
PROCEDURE

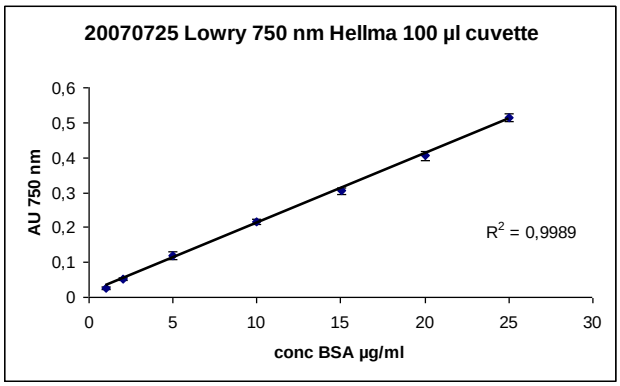
- **Preparation of assay:** Samples were diluted in 0.1 M NaOH up to 600 µl. Then 4 ml a mixture of solutions (96% reagent A, 2% reagent B and 2% reagent C) was added to each sample. Samples were left for incubation in RT for at least 10 minutes. Then 400 µl of a 1:1 dilution of Folin-Ciocalteu's phenol reagent in ddH_20 was added followed by immediate vortexing. The samples were left at RT for 1 hour before reading the absorbance.
- **700 µl cuvette:** Default flash and average settings applied. Between runs and concentrations, the cuvette was removed from the spectrophotometer to be washed before new concentration liquid is filled into it. Washing was performed by first squirting plenty of ddH_20 into the cell using a flask with a long tube to build up pressure. Afterwards, 70 % ethanol is squirted into the cell utilising a thin beam. The cell was then once more flushed with plenty of ddH_20. After the washing procedure, the cell was dabbed against dry tissues to remove excess fluid. The cell was wiped clean using lint free Kimwipes. 600 µl filled into the cuvette for

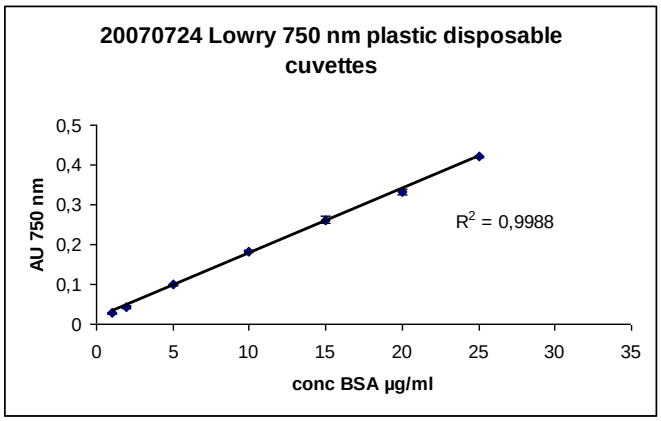
measurements.

- **100 µl cuvette**: same procedure as for 700 µl cuvette. Filled with 150 µl for measurements.
- **Plastic disposable cuvettes:** The cuvettes were filled up to 2/3, wiped clean using Kimwipes and then measured for absorbance.

RESULTS







REMARKS

- Position important for 100µl cuvette measurement.
- No liquid, lint or fingerprints must be on the smooth windows that are exposed to the light beam.
- Slowly empty the pipette into cuvette when measuring with the 100 µL cuvette in order to avoid build-up of bubbles in cuvette window.

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