Protein Quantification with Bradford Assay at 595 nm Measurement

MATERIAL

- BSA (Sigma-Aldrich Ltd., St Louis, MO; U.S.A.)1 mg/ml diluted in PBS.
- Standard curve with concentrations 1 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml and 25 µg/ml. Diluted in 0.01 M PBS pH 7.4.
- 1:5 Bio-Rad Protein assay (Bio-Rad, Hercules, CA, U.S.A.).
- SpectroArt 200, Protein Function, Bradford 595 nm, 5 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, disposable plastic 3 ml cuvettes 10 mm PL
- ddH_20 and 70 % ethanol for washing.
- Cotton buds for cleaning of TrayCell.

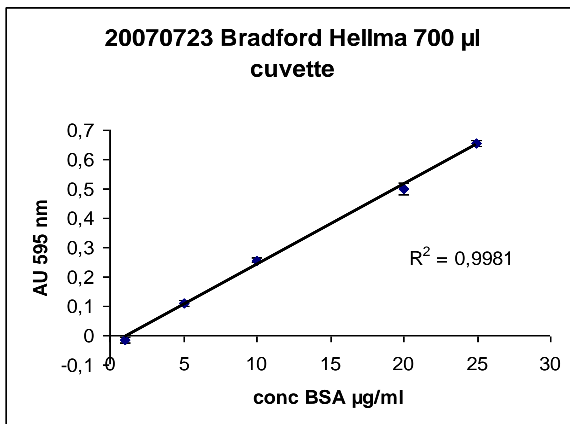
PROCEDURE

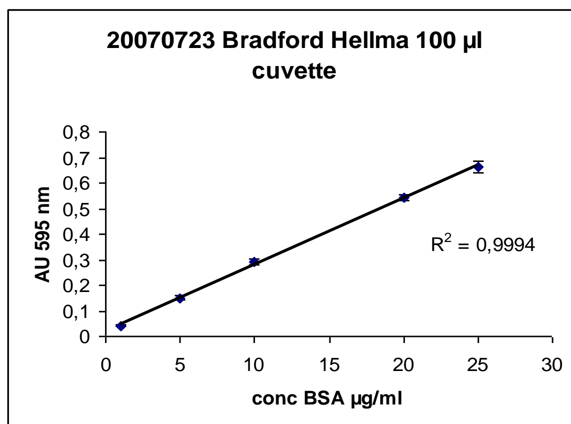
- **Assay procedure:** BSA was diluted in PBS to get the final concentrations used for the standard curve and then Bio-Rad protein assay was added (1:5) to all samples. The samples were incubated at RT before reading ABS at 595 nm.
- **700 µl cuvette:** Default flash and average settings were applied. Between runs and concentrations, the cuvette was removed from the spectrophotometer to be washed before new concentration liquid was filled into it. Washing was performed by first squirting ddH_20 into the cell, using a flask with a long tube to build up pressure. Then 70 % ethanol was squirted into the cell (thin narrow beam). After that, plenty of ddH_20 was again squirted into the cuvette to remove any ethanol residue. Finally, the cell was dabbed against tissues and then wiped clean using lint free Kimwipes. 600 µl was filled into the cuvette for measurements
- **100 µl cuvette**: same procedure as for 700 µl cuvette. Filled with 150 µl for measurements
- **TrayCell:** The cell was fitted into the spectrophotometer and flash-average settings were adjusted to maximum (8 flashes, 50 averages). To find the best obtainable position of the cell, the scanning function was used. The position that generated

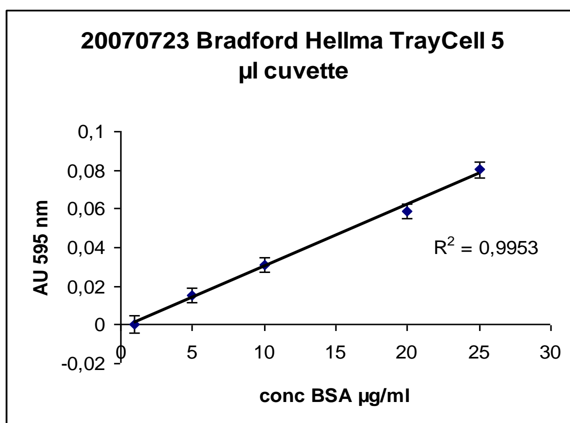
least noise when the TrayCell was empty was chosen and this position was kept throughout the measurement-series. After a measurement, the cap was removed and a cotton bud was used to collect the drop from the window and sample residue from the mirror. Another cotton bud was soaked in ddH_20, and used to wipe the mirror and the window clean of protein. The window and the mirror was then wiped dry and another cotton bud was soaked into 70 % ethanol and used to wipe the window and mirror clean of Bradford reagent. Window/mirror was dried clean with new cotton bud and the procedure was repeated with new ddH_20 and new cotton bud. Finally, the window and the mirror were dried clean before another sample was added. Measured: 5 µl

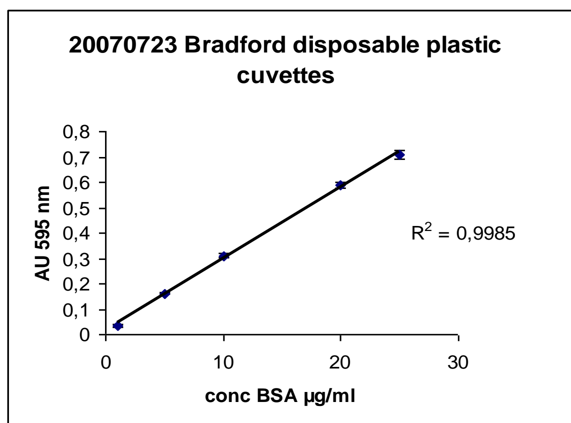
- **Disposable plastic cuvettes:** 3 ml solution is added to new clean disposable plastic cuvettes

RESULTS









REMARKS

- Bradford reagent sticks to quartz and glass, plastic disposable cuvettes are more suited for this assay. Measurements with quarts are OK, but excessive cleaning with both ddH_20 and ethanol is needed. It is important to remove any ethanol since this may interfere with absorbance readings.
- Position important for 100 µl and TrayCell cuvette measurement.
- No lint or fingerprints must be on the windows that are exposed to the light beam.
- Make sure no lint is present. Apply sample and place the cap on top (same position).

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