Protein Quantification at 280 nm Wavelength

MATERIAL

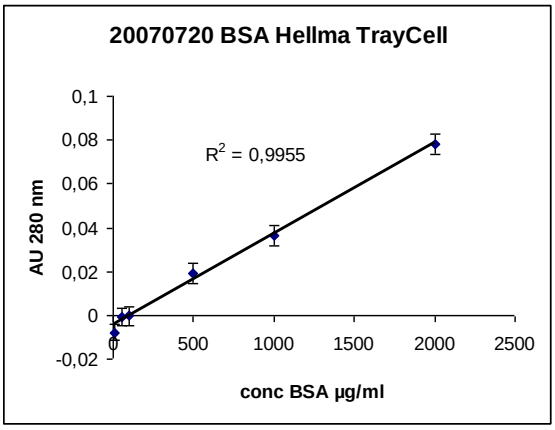
- BSA (Sigma-Aldrich Ltd., St Louis, MO, U.S.A.) 2 mg/ml diluted in 0.01 M PBS pH 7.4.
- Standard curve with concentrations 10 µg/ml, 50 µg/ml, 100 µg/ml, 500 µg/ml, 1000 µg/ml, 2000 µg/ml. Diluted in 0.01 M PBS pH 7.4.
- SpectroArt 200, Protein Function, customised wavelength 280 nm, 6 standard curve points, read blank 2 times and samples 5 times.
- Hellma™ cuvettes: 5 µl TrayCell with 1 mm PL cap, 100 µl black wall cuvette 10 mm PL, 700 quartz cuvette 10 mm PL.
- ddH_20 and 70 % ethanol for washing.
- Cotton buds for cleaning of TrayCell.

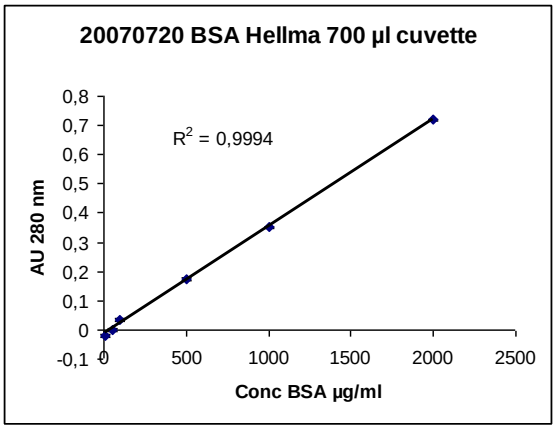
PROCEDURE

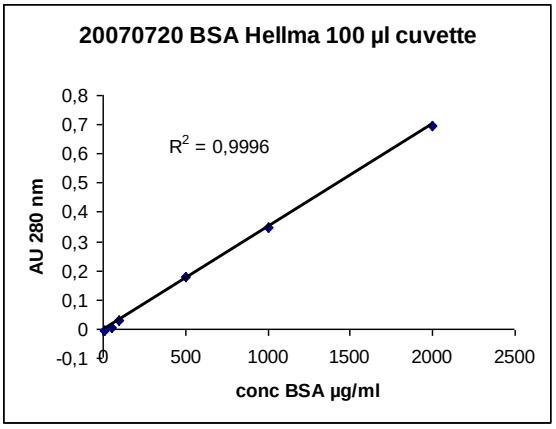
- **700 µl cuvette:** Default flash and average settings were applied. Between runs and concentrations, the cuvette was removed from the spectrophotometer to be washed before new concentration liquid was filled into it. Washing was performed by first squirting ddH_20 into the cell using a flask with a long tube to build up pressure. The cell was then emptied and 70 % ethanol was sprayed into the cell (thin jet) a couple of times. Afterwards, plenty of ddH_20 was squirted into the cell again to remove any ethanol residue. The cell was dabbed against tissues and then wiped clean using lint free Kimwipes. Measurements were performed on 600 µl.
- **100 µl cuvette:** same procedure as for 700 µl cuvette. Filled with 150 µl for measurements.
- **TrayCell**: The cell was fitted into spectrophotometer and flash/average-settings were adjusted to maximum (8 flashes, 50 averages). The best measurement position for the cell was tested using the scanning function. The position that generated the least noise with air in the cuvette was chosen and the TrayCell was kept still throughout the series of measurement. After a single measurement, the

cap was removed and a cotton bud was used to collect the drop from window and sample residue from the mirror. Then another cotton bud was soaked in ddH_20, and used for wipe the mirror and the window. The procedure was repeated with new water and a new bud. Finally, a new bud was used to wipe window/mirror dry and clean, making sure no lint was left on the mirror or the window. A new sample was applied and the cap was placed on top (same position). Measured with 5 µl.

RESULTS







REMARKS

- Cleaning and position important for TrayCell measurements. No lint must be left on window or mirror.
- Because of the virtual dilution factor of 10 for measurements with the TrayCell, the absorbance values obtained are approx. 10 times lower when measuring with the TrayCell. Very low concentration samples can therefore not be measured accurately with the TrayCell. A higher starting concentration is recommended for the absorbance values to reach above noise levels.
- Position important for 100µl cuvette measurement.
- No lint or fingerprints must be on the windows that are exposed to the light beam.

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