Bradford Assay

Materials:

- BSA standard solution (0.1 µg/µl)
- Bradford solution
    - Dissolve 100 mg Coomassie Brilliant Blue **G**-250 in 50 ml 95% ethanol. Add 100 ml of 85% phosphoric acid while stirring continuously. When the dye has dissolved, dilute to 1 l in H_2O. Filter to remove residual precipitate (Whatman paper) and store at 4 °C in a dark bottle.

Procedure:

1. Prepare protein standards according tot he following scheme

Name	STD 0	STD 2	STD 4	STD 6	STD 8	STD 10
H_2O	100µl	80µl	60µl	40µl	20µl	0µl
BSA	0µl	20µl	40µl	60µl	80µl	100µl

2. Prepare a 1:10 dilution of your protein sample (=SPL 10)
3. Prepare a dilution series of SPL10 according to the following scheme. For protein concentrations between 5-20 mg/ml it is sufficient to prepare SPL40-SPL200

Name	SPL 20	SPL 40		SPL 100	SPL 200	SPL 400		SPL 1000
H_2O	100µl	100µl		180µl	100µl	100µl		180µl
Sample	100µl SPL 10	100µl SPL 20		20µl SPL 10	100µl SPL 100	100µl SPL 200		20µl SPL 10
Dilution factor	20	40		100	200	400		1000

4. Transfer 10µl dublicates of each standard and sample into a 96-well plate and add 180µl Bradford reagent
5. Incubate 10min at room temperature and measure absorbance at 595nm
6. Prepare a calibration curve by plotting absorbance versus protein amount in µg
7. Determine the amount of protein in your samples using the trend line and the Lambert-Beer equation