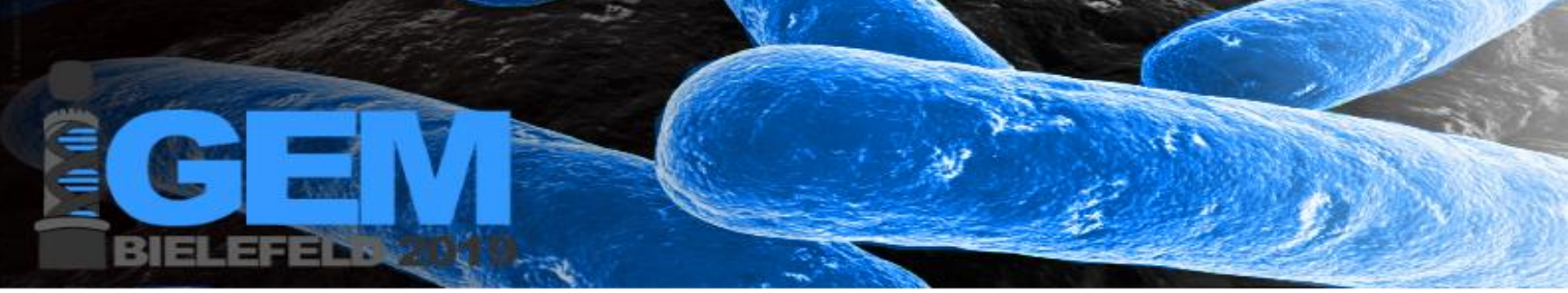

Bradford Assay with ROTI®-Nanoquant K880 from Carl Roth

For protein quantitation assay in cuvettes

1. Pipette the following volumes into clean cuvettes:
    - For zero value (sample 1) of the calibration line: 200 µl ddH_2O + 800 µl Roti®-Nanoquant working solution.
    - For calibration series (sample 2 to sample S): 200 µl of each standard + 800 µl Roti®-Nanoquant working solution.
    - For actual analysis (Sample T to Z): 200 µl of each sample + 800 µl Roti®-Nanoquant working solution.
2. Mix by inverting repeatedly
3. Pipette H_2O_{dd} in your reference cuvette.
4. Determine OD_{590} of all samples (sample 1 to Z) with ddH_2O as reference.
5. Determine OD_{450} of all samples (sample 1 to Z) with ddH_2O as reference.
6. Plot quotient OD_{590}/ OD_{450} and compare to amount of protein used. The protein amount in your sample corresponds to a certain value of the calibration line.

Lower detection limit: 0.2 µg protein (c = 1 µg/ml)

<u>Preparing the calibration standards:</u>

Prepare the calibration standards as follows. It is recommended to first pipette the standards in bold and prepare all other standards using these two stock solutions.

BSA (µg/ml)	µl (from dil.)	µl ddH_2O
0	-	200
1	20 µl (from 10 µg/ml)	180
2.5	50 µl (from 10 µg/ml)	150
5	100 µl (from 10 µg/ml)	100
10	40 µl (from 100 µg/ml)	360
25	50 µl (from 100 µg/ml)	150
50	100 µl (from 100 µg/ml)	100
75	150 µl (from 100 µg/ml)	50
100	200 µl (from 400 µg/ml)	600

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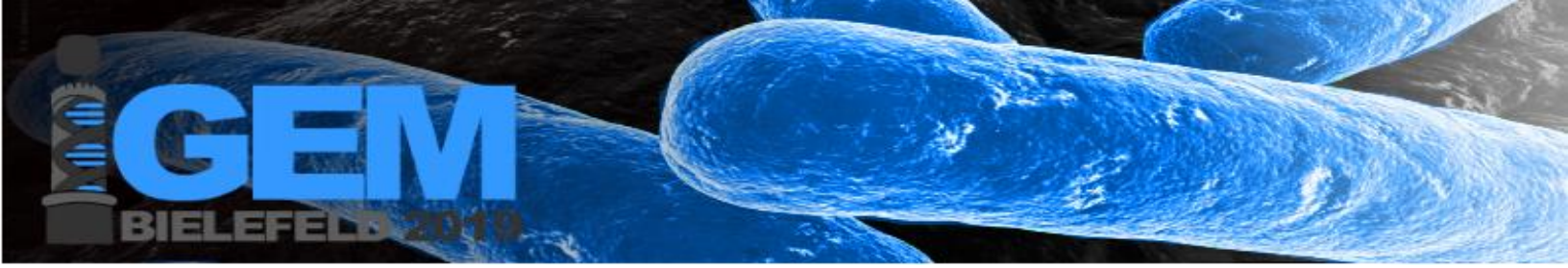

Roti®-Nanoquant Working Solution:

20 ml Roti®-Nanoquant + 80 ml ddH_2O

BSA-concentrations for calibration series

200 ng / 200µl = 1 µg / ml to 20 µg / 200 µl = 100 µg/ml

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