Instructions for use

ROTI®Nanoquant

Protein quantitation assay for low amount of protein

Storage temperature: +4 °C

A. Introduction

ROTI®Nanoquant solution has been established as modification of Bradford's protein assay (1, 2). Coomassie Brilliant Blue Dye-G250 appears in three different states which absorb at varying wavelengths (Figure 1).

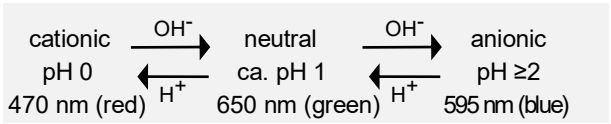
By binding the dye with a protein it changes from a cationic to an anionic state and its absorption level is 595nm. This absorption change is proportional to the protein concentration over a wide range, and it was first utilized in concentration analysis by Bradford (1).

Coomassie Brilliant Blue-G250 binds primarily to basic amino acids (2). This accounts for the difference in the level of absorption of varying proteins. For this reason, we highly recommend measuring the absorption level on a calibration curve using BSA.

By using ROTI®Nanoquant, reproducible protein amounts from 200 ng onwards (200 µl with c=1 µg/ml in cuvette assays, or 50 µl with c=4 µg/ml in 96well culture plates) can be analised in aqueous solutions.

Each sample is measured at 590 nm and 450 nm. Linearity results from the quotient $OD_{590/450}$.

Figure 1: Three absorbing states of CBBG 250 (3)



Slight variations in colour of the 1x-staining solution are dependend on the dye lot used and don't alter measurement results. In case of the stock or 1x solutions turning blue *prior to* mixing with proteins see Trouble shooting (H).

B. Formulas

ROTI®Nanoquant working solution:

20 ml ROTI®Nanoquant (5 times conc.)
+ 80 ml H_2O_{dd}

ROTI®Nanoquant working solution needs not be filtered. The working solution is stable for approx. one week at room temperature.

BSA-concentrations for calibration series:

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

C. Assay in cuvettes

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

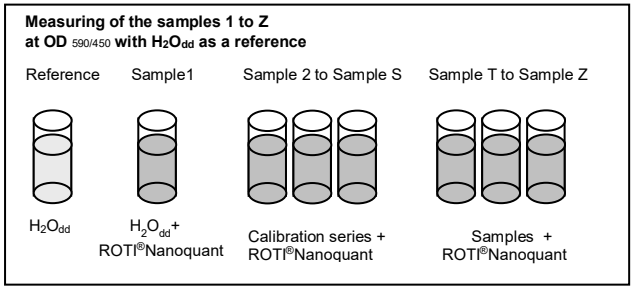


Figure 2

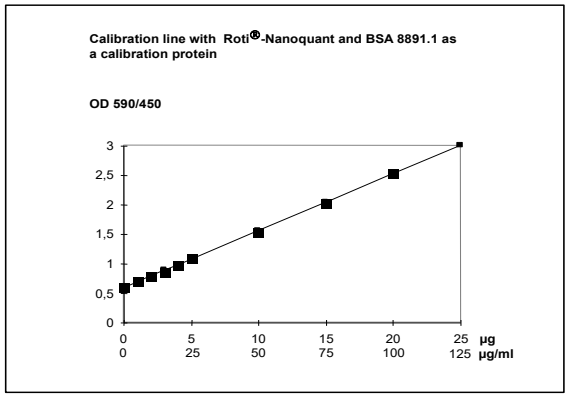


Figure 3 (cuvette assay)

D. Protein assay in 96well culture plates

1. Required equipment
   - 96-well culture plate
   - ELISA reader
   - BSA stock solution (400 µg/ml); We recommend using lyophilised Roth Albumins, e.g. Fraction V, Europe (Art. No. 1ETA), or Albumin solution 30 %, (Art. No. 9401)
2. Dilute the samples (s) to be measured, e.g.:

Dilution of sample	Pipetting schedule
1:20	10 µl P + 190 µl H_2O_{dd}
1:40	5 µl P + 195 µl H_2O_{dd}

3. According to your schedule pipette into the wells of your culture plate 50 µl of each calibration standard and of the sample dilutions. We recommend pipetting

each solution at least twice to verify your results within a double assay.

4. Dilute 1 volume ROTI®Nanoquant (5x) in 4 volumes H_2O_{dd} and pipette 200 µl of this solution to the standards and the samples on your plate.
5. Incubate the culture plate for 5 mins. at room temperature. Thereafter measure OD_{590} and OD_{450}.
6. Plot and compare the **quotient OD_{590}/OD_{450}** of each sample to the calibration curve.

Lower detection limit: 0.2 µg protein (c=4 µg/ml)

E. Calibration Standards

Prepare your calibration standards as follows. We recommend to first pipette the standard of 10 and 100 µg BSA/ml (bold lines), from albumin stock solution, and to prepare all other standards using these two solutions.

Pipetting schedule:

BSA (µg/ml)	µl (from dil.)	µl H_2O_{dd}
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**

F. Tips for better pipetting accuracy

1. Submerge the tip only a few mm into liquid.
2. Hold pipette vertically.
3. Wet tip with protein solution prior to sample removal.
4. Lean tip against the tube when releasing liquid.
5. Only use high-quality tips. We recomment Mµlti®-standard tips 1-200 µl, (Art. No. 7058.1) and 100-1000 µl (Art. No. 8163.1).

G. Interference with measurements

In general, protein quantitation acc. to Bradford is a method which reacts quite sensitive to reagents present. Due to the fact that ROTI®Nanoquant has been modified in order to enhance detection and increase sensitivity, hindrance caused by reagents has also been increased.

Bradford assays are known to work interference-free only in low concentrated salt solutions like 1 x PBS or similar phosphate bufferd systems. One may also use low concentrated Tris-buffered systems like 1 x TBS.

Please note: Proteins for standardisation of measures have to be solubilised in the same buffer system as samples.

Interference my particularly happen when dealing with samples containing detergents but also occurs with samples containing other substances, e.g. glycerol (see table).

	max. concentr. of incompatible substances
Triton X 100	0.025 %
SDS	0.005 %
Chaps	0.03 %
Desoxycholate	0.002 %
Glycerine	5 %

Overall, are recommend for quantitation of such samples the use of ROTI®Quant universal (Art. No. 0120). Based on a Biuret reaction, this assay is widely uneffected by inhibiting reagents.

H. Trouble shooting

x Assay results are not reproducible → Make sure to mix the stock solution prior to taking an aliquot.

x Stock sol. or 1x staining solution blue / zero value very high / curve very flat → Acidify solution with ortho-phosphoric acid until colour change.

Background: The Coomassie® dye may have one of three ionic conditions with characteristic colours (see also fig. 1 page 1)

cationic	red	pH ~0	470 nm
neutral	green	pH ~1	650 nm
anionic	blue	pH ≥2	595 nm

Only the *cations* have a strong protein binding efficiency, neutral or anionic forms of the dye bind only weakly. For efficient protein binding, the stock solution must therefore be coloured red-brown and the 1x dye solution red-brown to greenish-brown. If the stock solution or the 1x solution changes colour to **blue**, it should be acidified with a few drops of ortho-phosphoric acid so that the cationic ionic form predominates again.

I. References

(1) Bradford, M., (1976) *Anal. Biochem.* 72:248-254.
(2) Niess, U., (2004) *J Bacteriol* 186:3640-3648.
(3) Seipp, S., (2006) *Int. J. Dev. Biol.* 50:63-70.
(4) Zor, T. und Selinger, Z., (1996) *Anal. Biochem.* 236:302-308.

K. Safety information:

Danger H226-H290-H302-H314
P210-P280-P303+P361+P353-P305+P351+P338

ROTI®Nanoquant	**K880.2**	**50 ml**
	K880.3	**200 ml**
	K880.1	**500 ml**

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