Instructions for use

ROTI®Quant

Protein quantitation assay according to Bradford

Storage temperature: 4 °C

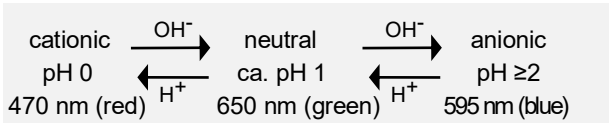
A. Introduction

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.

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 (F).

B. Macroassay (20-100 µg protein)

Dilute 1 volume of 5X-staining solution with 4 volumes redistilled H_20. Filter the diluted solution through a paper filter. The 1X-staining solution will be stable for approx. one week at room temperature.

Prepare your dilutions of the calibrating protein at a concentration of 0.2-1 mg/ml in sample buffer. When measuring samples, we recommend comparing them to the standard solutions.

1. Pipette 100 µl each of sample buffer, standard solutions and samples into clean test tubes.
2. Add 5 ml 1X-staining solution
3. Mix by inverting repeatedly.
4. Measure the OD_{595} of the standard solutions and samples after 5 to 30 mins against the reference (sample buffer in 1X-staining dye).
5. Plot and compare OD_{595} of standard solutions to amount of protein used. The amount of protein in sample can be read on calibration curve.

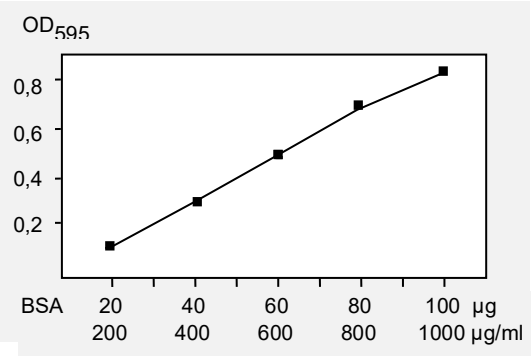


Figure 2 shows a typical standard curve with ROTI®Quant (macroassay) and BSA as calibrating protein.

C. Microassay (1-20 µg protein)

The 5X-solution is used directly in microassay. Diluting and filtering are not required.

Prepare your dilutions of the calibrating protein at a concentration of 1-20 µg/ml in sample buffer.

When measuring samples, we recommend comparing them to standard solutions in each assay.

1. Pipette 800 µl each of sample buffer (for zero value), standard solutions and samples into clean test tubes.
2. Add 0.2 ml 5X-staining solution.
3. Mix by inverting repeatedly.
4. Measure the OD_{595} of the standard solutions and samples after 5 to 30 mins against the zero value (0.8 ml 1X sample buffer + 0.2 ml 5X-staining dye).
5. Plot and compare OD_{595} of standard solutions to amount of protein used. Amounts of proteins in samples can be read on calibration curve.

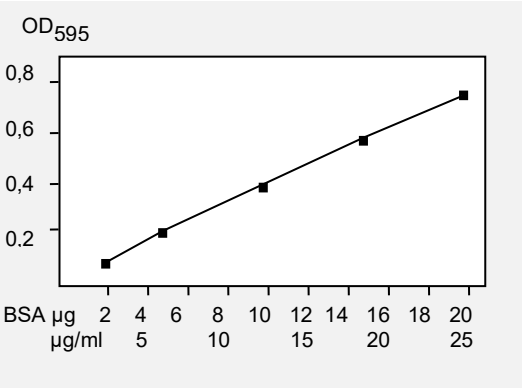


Figure 3 shows a typical standard curve with ROTI®Quant (microassay) and BSA as calibrating protein.

D. Protein assay in 96-well 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. Please prepare your calibration standards at a concentration of 20-100 µg/ml. We recommend to prepare the 100 µg/ml solution first, and, subsequently, mix the other concentrations simply by dilution.

Pipetting schedule:

BSA (µg/ml)	µl BSA-solution	µl H_2O_{dd}
0	-	110
20	40 µl out of 100 µg/ml	160
30	45 µl out of 100 µg/ml	105
40	80 µl out of 100 µg/ml	120
50	60 µl out of 100 µg/ml	60
60	120 µl out of 100 µg/ml	80
80	160 µl out of 100 µg/ml	40
100	200 µl out of **400** µg/ml	600

3. Please dilute the samples to be measured:

Example:

Dilution of samples	Pipetting schedule
1:20	10 µl s. + 190 µl H_2O_{dd}
1:40	5 µl s. + 195 µl H_2O_{dd}

4. According to your schedule pipette 50 µl of each calibration standard and of the diluted sample into wells of your culture plate. We recommend measuring each solution at least twice in order to verify your results by a double assay.

5. Please dilute 2 volumes ROTI®Quant (5x) in 5.5 volumes H_2O_{dd} and add 200 µl of this solution to the standards and the samples on your plate.

6. Incubate the culture plate for 5 min at room temperature. Then measure OD_{595}.

7. Plot and compare OD_{595} of each sample to the calibration curve.

E. Compatibility

Due to the high dilution of the sample and the reagents contained within, the macroassay is essentially compatible with many chemicals.

A reduction in the extent of sensitivity in micro-assay however, can arise. This is particularly the case when dealing with samples containing detergents but also occurs with samples containing other substances, e.g. glycerol (Table 1).

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. If, however, purification of samples from incompatible reagents is required, please follow the intructions given here (4):

Following solutions are required:

500 mM KPh pH 7.4, 250 mM $CaCl_2$ Ethanol pA

Sample pH should be neutral.

1. Add 20 µl 500 mM KPh, pH 7.4 to 400 µl of sample and mix by inverting 3 times.

2. Then add 20 µl 250 mM $CaCl_2$ and mix again by inverting 3 times.

3. Finally pour in 1 ml ethanol and thoroughly mix the suspension.

4. Centrifuge for 1 minute, 7000 g and remove supernatant.

5. Now add 100 µl H_2O and 1 ml ethanol. Mix thoroughly.

6. Centrifuge suspension for 1 minute, 7000 g and remove supernatant.

7. Add 200 µl 5X-staining solution and wait for 5 minutes. Add 800 µl water and measure OD_{595}.

Table 1: Compatible concentration of reagents (400 µl sample/microassay)

	without sample preparation	with sample preparation
Triton X 100	0.025 %	3 %
SDS	0.005 %	0.33 %
Chaps	0.03 %	2.5 %
Desoxycholate	0.002 %	0.075 %
Glycerine	5 %	30 %

F. 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.

G. References

(1) Bradford (1976), *Anal. Biochem.* 72:248-254.
(2) Chial und Splittgerber (1993), *Anal. Biochem.* 213:362-369.
(3) Compton und Jones (1985), *Anal. Biochem.* 151:369-374.
(4) Pande und Murthy (1994), *Anal. Biochem.* 220:424-426.

H. Hazards and Precautionary Phrases

Danger H226-H290-H302-H314

P210-P280-P303+P361+P353-P305+P351+P338

ROTI®Quant	**K015.2**	50 ml
	K015.3	200 ml
	K015.1	500 ml

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