INSTRUCTION MANUAL

Bradford Reagent, 5x

Reagent for protein quantification
(Cat. No. 39222)

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Inhaltsverzeichnis

1.   Bradford Assay

1.1.   General Information

The Bradford assay is the mostly used colorimetric assay for protein quantification. The assay bases on the shift of the Coomassie® absorption maximum from 470 nm to 595 nm after protein binding at acidic pH (Fig. 1). The protein concentration of an unknown sample can be determined with the help of a calibration curve. The colorimetric reaction depends on the content of aromatic and basic amino acids. To create the calibration curve, it is recommended to use bovine serum albumin (BSA) as standard.
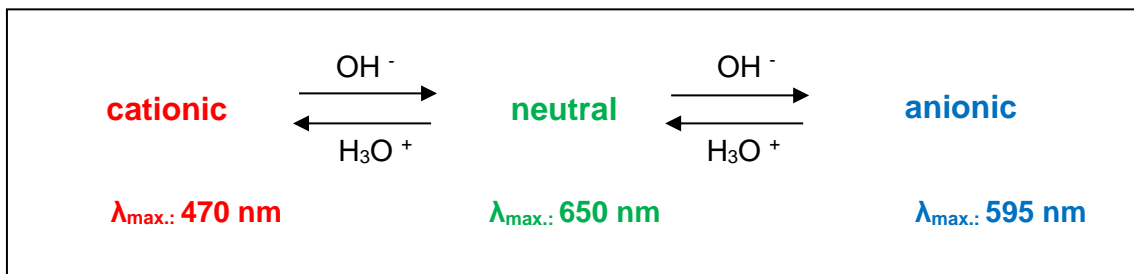

Figure 1: pH dependent absorption of Coomassie® Brilliant Blue G 250

Advantages of the method:

- Fast assay procedure

- Very sensitive

- Protein specific

- Stable dye protein complexes

Coomassie® = registered trademark of *ICI Ltd.*

1.1. Additionally required equipment

- Centrifuge suitable for centrifugation of 1.5 ml and 15 ml sample tubes at 12,000xg

- Vortex mixer

- Photometer suitable for measurement at 595 nm wavelength and the usage of microcuvettes, e. g. ELISA-Reader for microtiter plate assay

- Plastic cuvettes (**Please do not use quartz cuvettes because the dye would bind to this material.**), microtiter plates

1.2. Storage conditions

The recommended storage temperature for the Bradford Reagent is +2 °C to +8 °C. Under these storage conditions the unopened reagent is at least useable until: see expiry date on the label.

2. Performance of the protein determination according to Bradford

2.1. Micro assay (1 - 25 µg/ml Protein)

2.1.1. Preparation of solutions

For the Bradford micro assay the 5x Bradford Reagent is used undiluted. Please mix the reagent gently by inverting the bottle several times. (Do not shake the bottle to mix the solution!). Remove the amount of reagent needed and equilibrate it to room temperature before use.

To create the calibration curve the reference protein should be diluted as follows: 5, 10, 15, 20, 25 µg/ml. The assay is performed as triplicate determination. The calibration curve should be created new for each series of tests.

2.1.1. Preparation of reference solutions

The pipetting scheme for the preparation of the reference solutions for micro determinations can be found in Table 1. As diluent for the preparation of the calibration curve, the buffer should be used in which the protein sample to be determined is also present.

Table 1: Pipetting scheme for preparing the reference solutions for micro determination.

Concentation reference protein (e.g. BSA) [µg/ml]	Volum stock solution (1 mg/ml) [µl]	Volume diluent [µl]
0	0	10
1	10	9.990
5	50	9.950
10	100	9.900
15	150	9.850
20	200	9.800
25	250	9.750

2.1.2. Protein quantification procedure

Protein determination is performed in a 3-fold determination.

Pipet **800 µl** dist. H_2O and buffer for blank, reference
solutions and samples into test tubes

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Add **200 µl 5x Bradford reagent**

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Close test tubes

↓

Vortex test tubes

↓

Incubate **5 min** at RT

↓

Transfer solutions to cuvettes
Measure absorbance at 595 nm (A_{595nm})
within 30 min

2.1.3. Calculation of protein concentration

Create a table with the absorbance results obtained from the assay. From the values obtained for the reference protein solutions create a calibration curve which is used to determine the protein concentration in the unknown sample. Table 2 shows exemplary absorbance results for the creation of a calibration curve with BSA (samples were dissolved or diluted in dist. H_2O). Figure 2 shows the resulting BSA calibration curve.

Table 2: Example of assay data table of BSA reference solutions

c(BSA) [µg/ml]	1	5	10	15	20	25
$A_{595\ nm}$	0,066 0,068 0,067	0,224 0,225 0,222	0,402 0,410 0,406	0,573 0,569 0,570	0,720 0,705 0,710	0,851 0,892 0,860

The calculation is made with linear regression of the reference solutions and the following conversion of the absorption values of the sample solutions in protein concentrations through the regression equation.
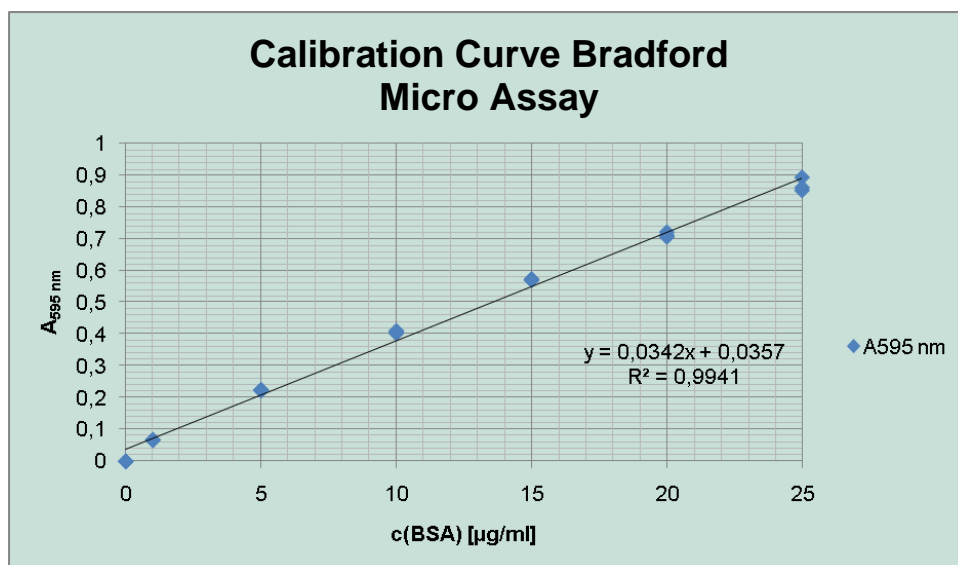

Figure 2: BSA calibration curve produced from the assay data in table 1. This standard curve was produced using BSA as a standard in triplicate points. The data are fit with linear regression by the line y = 0.0342x + 0.0357 with R^2 value of 0.9941. c = concentration

Note:

The data below should not be used as a replacement of a calibration curve. The absorbance of the BSA reference solutions in each assay will differ from those presented here.

2.2. Macro assay (100 -1000 µg/ml protein)

2.2.1. Preparation of solutions

Please mix the reagent gently by inverting the bottle several times. (Do not shake the bottle to mix the solution!). Remove the amount of reagent needed and equilibrate it to room temperature before use. To perform the macro assay, dilute the 5x Bradford Reagent 1:5 (1 part by volume plus 4 parts by volume H_2O bidest.) and filter the solution. The 1x Bradford solution could be stored for 1 week at RT.

To create the calibration curve the reference protein should be diluted as follows: 100, 125, 250, 500, 1000 µg/ml. The assay is performed as triplicate determination. The calibration curve should be created new for each series of tests.

2.2.2. Preparation of reference solutions

The pipetting scheme for the preparation of the reference solutions for macro determinations can be found in Table 3. As diluent for the preparation of the calibration curve, the buffer should be used in which the protein sample to be determined is also present.
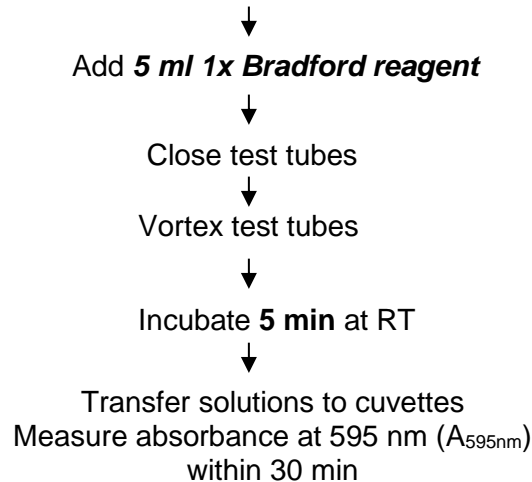
Table 3: Pipetting scheme for preparing the reference solutions for macro determinations.

Concentration reference protein (e.g. BSA) [µg/ml]	Volume stock solution (1 mg/ml) [µl]	Volume diluent [µl]
0	0	500
100	50	450
125	63	437
250	125	375
500	250	250
1000	500	0

2.2.3. Protein quantification procedure

Protein determination is performed in a 3-fold determination.

Pipet **100 µl** dist. H_2O and buffer for blank, reference
solutions and samples into test tubes

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Add **5 ml 1x Bradford reagent**

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Close test tubes

↓

Vortex test tubes

↓

Incubate **5 min** at RT

↓

Transfer solutions to cuvettes
Measure absorbance at 595 nm (A_{595nm})
within 30 min

2.2.4. Calculation of protein concentrations

Create a table with the absorbance results obtained from the assay. From the values obtained for the reference protein solutions create a calibration curve which is used to determine the protein concentration in the unknown sample. Table 4 shows exemplary absorbance results for the creation of a calibration curve with BSA (samples were dissolved or diluted in dist. H_2O). Figure 3 shows the resulting BSA calibration curve.

Table 4: Example of assay data table of BSA reference solutions

c(BSA) [µg/ml]	100	125	250	500	1000
$A_{595\,nm}$	0.161	0.190	0.359	0.587	0.896
	0.166	0.193	0.384	0.583	0.902
	0.164	0.192	0.369	0.593	0.892

The calculation is made with polynomial regression of the reference solutions and the following conversion of the absorption values of the sample solutions in protein concentrations through the regression equation. c = concentration
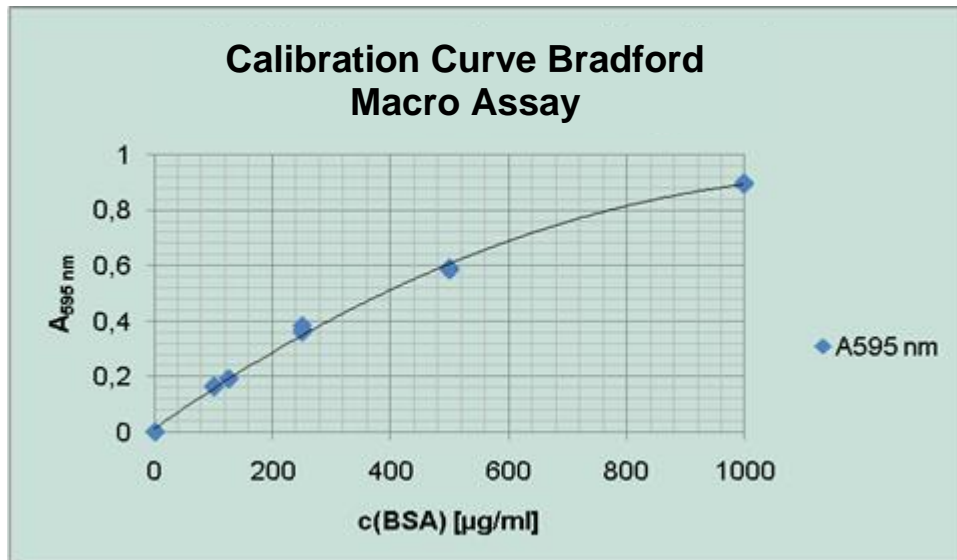
Figure 3: BSA calibration curve produced from the assay data in table 2. This standard curve was produced using BSA as a standard in triplicate points. The data are fit with polynomial regression by the line $y = -5*10^{-7}x^2 + 0,0014x + 0,0205$ with R^2 value of 0.9979. c = concentration

Note:

The data below should not be used as a replacement of a calibration curve. The absorbance of the BSA reference solutions in each assay will differ from those presented here.

2.3. Protein quantification using microtiter plates

2.3.1. Preparation of solutions

Please mix the reagent gently by inverting the bottle several times. (Do not shake the bottle to mix the solution!). Remove the amount of reagent needed and equilibrate it to room temperature before use. To perform the assay in microtiter plates, dilute the 5x Bradford Reagent 2:7,5 (2 parts by volume plus 5,5 parts by volume H_2O bidest.).

To create the calibration curve the reference protein should be diluted as follows: 20, 30, 40, 50, 60, 80 and 100 µg/ml. The assay is performed as triplicate determination. The calibration curve should be created new for each series of tests.

2.3.2. Preparation of reference solutions

The pipetting scheme for the preparation of the reference solutions for microtiter plates can be found in Table 5. As diluent for the preparation of the calibration curve, the buffer should be used in which the protein sample to be determined is also present.

Table 5: Pipetting scheme for preparing the reference solution for microtiter plates.

Concentration reference protein (e.g. BSA) [µg/ml]	Volumen Stock solution (100 µg/ml) [µl]	Volumen Diluent [µl]
0	0	200
20	40	160
30	60	140
40	80	120
50	100	100
60	120	80
80	160	40
100	200	0

2.3.3. Sample dilution

Please dilute the sample before testing.
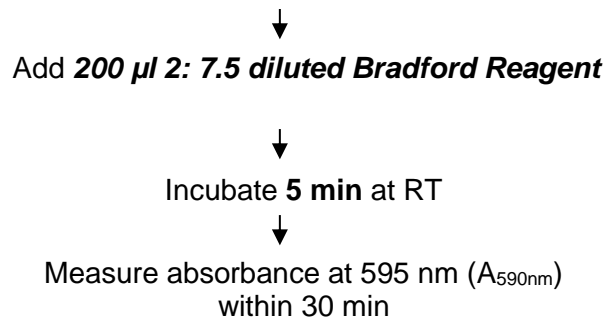
Example:
1:20 dilution: 10 µl sample + 190 µl diluent

1:40 dilution: 5 µl sample + 195 µl diluent

2.3.4. Protein quantification procedure

Protein determination is performed in a 3-fold determination.

Pipet **50 µl** dist. H_2O and buffer for blank, reference
solutions and samples into the wells of the microtiter plate

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Add ***200 µl 2: 7.5 diluted Bradford Reagent***

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Incubate **5 min** at RT

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Measure absorbance at 595 nm (A_{590nm})
within 30 min

2.3.5. Calculation of protein concentrations

Create a table with the absorbance results obtained from the assay. From the values obtained for the reference protein solutions create a calibration curve which is used to determine the protein concentration in the unknown sample.

3. Literatur

- **Bradford, M.M.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72: 248-254.

- **Compton, S.J. and Jones, C.J.** Mechanism of dye response and interference in the Bradford protein assay. Anal. Biochem. 1985; 151: 369-374.

- **Davies, E.M. Protein assays:** A review of common techniques. Amer. Biotech. Lab. 1988; July 28-37.