Bradford Protein Quantification kit

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Bradford Protein Quantification kit
#D05078.2000 dtn

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma

Fabriqué en France
Made in France

#D11078
Version: 0117

Table of contents

2000 determinations
Storage: +4°C
Expiry date: stated on the package

This kit contains:

Designation	Item #	Quantity per kit	Form
Bradford Protein Quantification Reagent	D22078.2000 dtn	1	Liquid
Bradford Protein Quantification Standard	D06078.1 ea	2	Liquid
Technical Booklet	D11078.1 ea	1	-

Each kit contains sufficient reagents for:

- 2000 tests in 96-well plate. This allows for the construction of one standard curve in duplicate and the assay of 992 samples in duplicate (sample volume of 5 µL).
- 500 tests in 2 mL tubes. This allows for the construction of one standard curve in duplicate and the assay of 242 samples in duplicate (sample volume of 1 mL).

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area in which kit reagents are handled
- Avoid splashing

Wearing gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

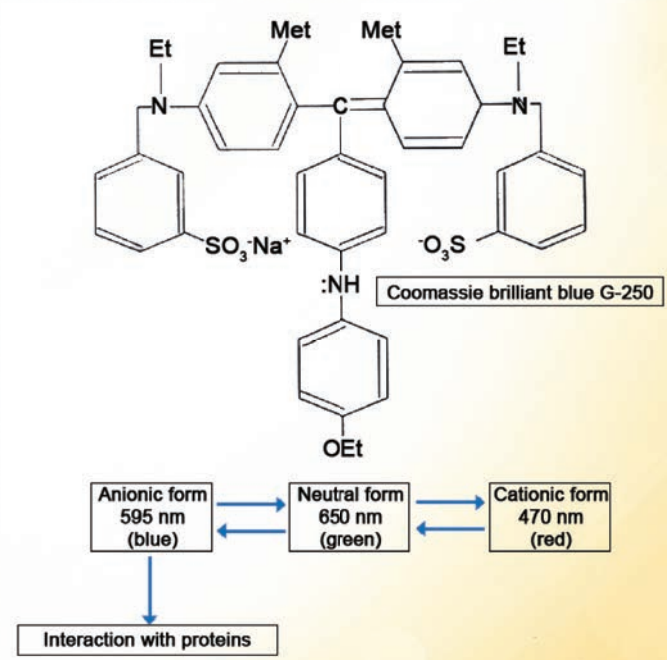
Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Background

The protein quantification method using Coomassie Brilliant Blue G-250 dye has been described for the first time in 1975 ***[1]***.

Three charge forms of the Coomassie Brilliant Blue dye are present in equilibrium at the usual acidic pH of the assay. The red, blue, and green forms have absorbance maxima at 470, 595, and 650 nm, respectively ***[2]***.



The red form donates its free electron to the protein.

Then the blue form interacts with the arginine, lysine, tryptophan, tyrosine, histidine and phenylalanine residues resulting in the stabilization of the blue form of the Coomassie Brilliant Blue G-250 dye. ***[3]***

The blue anionic form is negligible at the pH of the assay reaction mixture, the binding to protein shifts the equilibrium toward its formation.

The stabilized blue form of the Coomassie dye is measurable by spectrophotometry at 595 nm, the intensity of the coloration is directly linked to the amount of proteins.

The amount of proteins will be determined by comparaison of the absorbance at 595 nm obtained with a know concentration of protein solutions.

Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

For the microplate assay:

- Precision micropipettes & multichannel pipettes (10-200 µL)
- Disposable tips
- 96-well plates
- Orbital microplate shaker
- Microplate reader (595 nm filter)

For the microtube assay:

- Precision pipettes (100-5000 µL)
- Disposable tips
- Polypropylene microtubes
- Spectrophotometer (595 nm filter).

Sample collection and preparation

No particular attention for the collection and preparation of the samples.

Pay attention to the pH of your sample as it could affect the reaction. Highly alkaline buffers present interference, in this case, use another method for the protein quantification like BCA.

Find hereafter a table of reagents compatible with this kit when the standard procedure is used.

This is not a complete compatibility chart. There are many substances that can affect different proteins in different ways.

One may assay the protein of interest in deionized water alone, then in buffer with possible interfering substances.

Comparison of the readings will indicate if an interference exists. Refer to the literature for additional information on interfering substance ***[4]***.

These concentration limits are compatible for the microtube procedure, when the microplate assay is used, concentration limits are 1/25 of the values in the Table.

Substance	Compatible concentration
Buffers and additives	
Eagle´s MEM	Undiluted
Earle´s salt solution	Undiluted
Glucose	20 %
Glycerol	5 %
Glycine	100 mM
Guanidine-HCl	2 M
Hank´s salt solution	Undiluted
Hydrochloric acid	100 mM
HEPES	100 mM
Imidazole	200 mM
Magnesium chloride	Undiluted
MES	100 mM
MOPS pH 7.2	100 mM
Modified Dulbecco´s PBS	Undilued
PBS	Phosphate 0.1 M-NaCl 0.15 M
Phenol red	0.5 mg/mL
PIPES	200 mM
PMSF	2 mM
Potassium chloride	2 M
Potassium phosphate	500 mM
SB 3-10	0.1 %
Sodium acetate pH 4.8	200 mM
Sodium azide	0.5%
Sodium bicarbonate	200 mM
Sodium Carbonate,	100 mM
Sodium Chloride	2.5 M
Sodium citrate pH 4.8 or pH 6.4	200 mM
Sodium hydroxide	100 mM

Substance	Compatible concentration
Buffers and additives (continue)	
Sodium phosphate	500 mM
Sucrose	10%
TBP	5 mM
TBS pH = 7.6-0.5 X	12.5mM Tris, 75 mM NaCl, pH 7.6
TCEP	20 mM
Thio-urea	1M
Tricine pH 8.0	50 mM
Triethanolamine, pH 7.8	50 mM
Tris	1 M
Tris-glycine	25 mM Tris, 192 mM glycine
Tris-glycine-SDS 0.5 X	12.5 mM Tris,96 mM glycine, 0.05 % SDS
Urea	4 M

Substance	Compatible concentration
Chelating agents	
EDTA	200 mM
EGTA	200 mM

Substance	Compatible concentration
Detergents	
CHAPSO	10 %
Deoxycholic acid	0.2 %
Octyl β-thioglucopyranoside	1 %
SDS	0.025 %
TRITON® X-100	0.05 %
TWEEN® 20	0.01 %

Substance	Compatible concentration
Reducing and thiol containing reagents	
Dithioerythritol (DTE)	10 mM
Dithiothreitol (DTT)	10 mM

Substance	Compatible concentration
Solvents	
DMSO	5 %
Ethanol	10%
Methanol	10 %

Reagent preparation

Bradford protein Quantification Standard

Mix the Bradford Protein Quantification Standard a few times before use.

	Microtube		Microplate		
Sample	Standard (µL)	Diluent (µL)	Standard (µL)	Diluent (µL)	Protein (µg / µL)
S8	1000	1000	100	100	5.00
S7	800	1200	80	120	4.00
S6	600	1400	60	140	3.00
S5	400	1600	40	160	2.00
S4	200	1800	20	180	1.00
S3	100	1900	10	190	0.50
S2	50	1950	5	195	0.25
S1 (Blank)	-	2000	-	200	0.00

Stability at 4°C: use within the day.

Assay procedure

It is recommended to perform the assays in duplicate following the instructions hereafter.

Plate set-up

A plate set-up is suggested hereafter for the microplate procedure.
The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33
B	S2	S2	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34
C	S3	S3	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
D	S4	S4	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
E	S5	S5	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
F	S6	S6	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
G	S7	S7	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39
H	S8	S8	C8	C8	C16	C16	C24	C24	C32	C32	C40	C40

S1-S8 : Standards C1-C40: Samples

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet all the reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip. Start with the lowest concentration standard (S0) and equilibrate the tip in the next higher standard before pipetting.

	Volume in µL			
	Microtube		Microplate	
	Standard	Samples	Standard well	Sample well
Standard	1000	-	5	-
Sample	-	1000	-	5
Bradford Protein Quantification Reagent	1000		250	
Mix gently 5 seconds with a vortex or an orbital shaker				
Incubate at room temperature for at least 5 min, maximum 1 hour.				
Read the absorbance at 595 nm optional: you may zero the spectrophotometer with the Blank.				

Data analysis

> If the spectrophotometer or the microplate reader were not zeroed with the Blank, subtract the average blank values to the absorbance readings of the rest of the plate.

> Create a standard curve by plotting A595nm (y-axis) vs standard concentration (x-axis).
Determine the unknown sample concentration using the standard curve.

> The level of detection of the assay is lower for the microplate assay when compared with the microtubes due to a shorter light path used in the microplate reader.

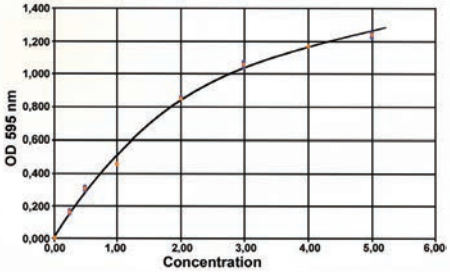
Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.

These data were obtained using all reagents as supplied in this kit with the microplate procedure developing at room temperature, reading at 595 nm. A 4-parameter logistic fitting was used to determine the concentrations.

Standard	[Standard] µg / µL	Absorbance A.U.
S8	5.00	1.239
S7	4.00	1.166
S6	3.00	1.057
S5	2.00	0.850
S4	1.00	0.456
S3	0.50	0.307
S2	0.25	0.168
S1	0.00	0.000

Typical Bradford standard curve



Troubleshooting

> **Absorbance of Blank is OK, but remaining standards and samples yield lower values than expected:**

- Improper reagent storage.
  Solution: Store reagent at +4°C

- Reagent is too cold.
  Solution: Allow Reagent to warm to RT

- Absorbance is measured at incorrect wavelength.
  Solution: Measure absorbance near 595nm

> **Absorbances of Blank and standards are OK, but samples yield lower values than expected**:

- Sample protein (peptide) has a low molecular weight (e.g., less than 3000).
  Solution: Use the BCA

> **A precipitate forms in all tubes**:

- Sample contains a surfactant (detergent).
  Solution: Dialyze or dilute sample

- Sample not mixed well or left to stand for extended time, allowing aggregates to form with the dye.
  Solution: Mix samples immediately prior to measuring absorbance

- **All tubes (including Blank) are dark purple**:
  - Strong alkaline buffer raises pH of formulation or
  - Sample volume too large thereby raising reagent pH.
    Solution: Dialyze or dilute sample

These are a few examples of troubleshooting that may occur.

If you need further explanation, Bertin Pharma will be happy to assist you. Feel free to contact our technical support staff by phone (+33 (0)139 306 036), fax (+33 (0)139 306 299) or E-mail (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

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2. Chial H.J., Thompson H.B., Splittgerber A.G.
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Mechanism of Coomassie brilliant blue G-250 binding to proteins: A hydrophobic assay for nanogram quantities of proteins.
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	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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