Fast Protein Quantification Assay kit (Bradford)

KB-03-003

2000/4000 tests (96 well plate)

BQCkit

A brand of Bio QuChem

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All chemicals should be handled with care

- This kit is for R&D use only

Introduction

The Bradford assay, a colorimetric protein assay, is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250.

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, 590, and 650 nm, respectively.

The blue is the form that binds the protein, forming a complex that intensely absorbs light at 595 nm. During the formation of this complex, two types of bond interaction take place: the red form of Coomassie dye first donates its free electron to the ionizable groups on the protein, which causes a disruption of the protein's native state, consequently exposing its hydrophobic pockets. These pockets in the protein's tertiary structure bind non-covalently to the non-polar region of the dye via van der Waals forces, positioning the positive amine groups in proximity with the negative charge of the dye. The bond is further strengthened by the ionic interaction between the two.

The binding of the protein stabilizes the blue form of the Coomassie dye; thus the amount of the complex present in solution is a measure for the protein concentration, and can be estimated spectrophotometrically.

Materials

BQCkitFast Protein Quantification kit *KB03003-2000 tests* contains:

Product	Quantity	Storage
Bradford Reagent	1 bottle	RT
Protein Standard*	2 vials	4ºC

*This Reagent is stable during 10 days at Room Temperature and is shipped in these conditions. Once received is recommended to keep it at 4ºC.

BQCkit Fast Protein Quantification kit *KB03003-4000 tests* contains:

Product	Quantity	Storage
Bradford Reagent	2 bottles	RT
Protein Standard*	4 vials	4ºC

*This Reagent is stable during 10 days at Room Temperature and is shipped in these conditions. Once received is recommended to keep it at 4ºC.

Assay Principle

The Bradford assay is based on the direct binding of Coomassie brilliant blue G-250 dye to proteins at arginine, tryptophan, tyrosine, histidine and phenylalanine residues. Anionic dye binds to these residues producing an absorbance maximum at 595 nm.

The dye interacts with the protein via van der Waals forces between the hydrophobic regions of the protein and the non polar region of the dye, and the electrostatic interaction between the negatively charged dye and positively charged amino groups of protein. The formation of the dye-protein complex takes about two minutes and remains stable for an hour, so the procedure is very fast and the time for trial is not limiting. The dye-protein complex has a high extinction coefficient, which makes the assay highly sensitive.

Highly alkaline buffers present interference but it can be countered running appropriate targets. Reagents as magnesium chloride, potassium, sodium, ammonium sulfate and ethanol have no effect on the assay.

The presence of large amounts of detergents has a high interference.

Reagents commonly used are listed in Table 1.

Assay Principle

Table 1. *Reagents compatible with the kit protein assay when the standard procedure is used**

Chapso, 10%
Deoxycholic acid, 0.2%
DMSO, 5%
Dithioerythritol(DTE), 10 mM
Dithiothreitol (DTT), 10 mM
Eagle´s MEM
Earle´s salt solution
EDTA, 0.2 M
EGTA 0.2 M
Ethanol, 10%
Glucose, 20%
Glycerol, 5%
Glycine, 0.1M
Guanidine-HCl, 2M
Hank´s salt solution,
HCl, 0.1M
HEPES 0.1M
Imidazole 0.2M
Magnesium chloride
MES, 0.1M
Methanol 10%
Modified Dulbecco´s PBS
MOPS, 0.1M
Triethanolamine, pH 7.8, 50mM
Tris, 1M
Tris-glycine (25mM Tris, 192mM glycine)
Tris-glycine-SDS (25mM Tris,192mMglycine, 0.1%SDS),0.5x
Octyl β-thioglucopyranoside, 1%
PBS
Phenol Red, 0.5 mg/mL
PIPES, 0.2 M
PMSF, 2 mM
Potassium chloride, 2 M
Potassium phosphate, 0.5 M
SB 3-10, 0.1%
SDS 0.025%
Sodium acetate, pH 4.8, 0.2 M
Sodium azide, 0.5%
Sodium bicarbonate, 0.2 M
Sodium Carbonate, 0.1 M
Sodium Chloride, 2.5 M
Sodium citrate pH4.8 or6.4,0.2 M
Sodium Hydroxide, 0.1M
Sodium Phosphate, 0.5M
Sucrose, 10%
TBP 5mM
TBS (25mM Tris, 0.15M NaCl, pH 7.6), 0.5x
TCEP, 20mM
Thio-urea, 1M
Tricine, pH 8, 50mM
Triton® X-100, 0.05%
Tween® 20, 0.01%
Urea, 4M

*These concentration limits are compatible for macroassay, when microassay is used, concentration limits are 1/25 of the values in Table 1.

Note: This is not a complet 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.

Assay Protocol

There are two protocols to perform the assay. The microassay is for samples with low protein concentrations. The 96 well plate assay is for those who wish to perform the Bradford assay in plate format.

If another format is required, extrapolate the volume of samples and reagents.

BQC Fast Protein Quantification Kit Micro assay (2 mL)

1. Invert the Bradford Reagent a few times before use.
2. Preparation of standards: Refer to the Table 2 as a guide for diluting the protein standard. For the diluent, use the same buffer as in the samples.

Table 2. *Microassay Standard Dilutions*

Sample	Standard [mL]	Diluent [mL]	Protein [mg]
S1(Blank)	---	2	---
S2	0.05	1.95	0.25
S3	0.1	1.9	0.5
S4	0.2	1.8	1
S5	0.4	1.6	2
S6	0.6	1.4	3
S7	0.8	1.2	4
S8	1	1	5

3. Pipette 1 mL of each standard or unknown sample solution into separate clean test tubes.

4. Add 1 mL of Bradford Reagent to each tube and vortex.
5. Incubate at room temperature for at least 5 min. Samples should not be incubated longer than one hour at room temperature.
6. Measure the absorbance of these standards, blanks and unknown samples at 595 nm.

BQC Fast Protein Quantification Kit Microplate

1. Invert the Bradford Reagent a few times before use.
2. Preparation of standards: Refer to the Table 3 as a guide for diluting the protein standard. For the diluent, use the same buffer as in the samples.
3. Pipette 5 µL of each standard or unknown sample solution into microplate wells.

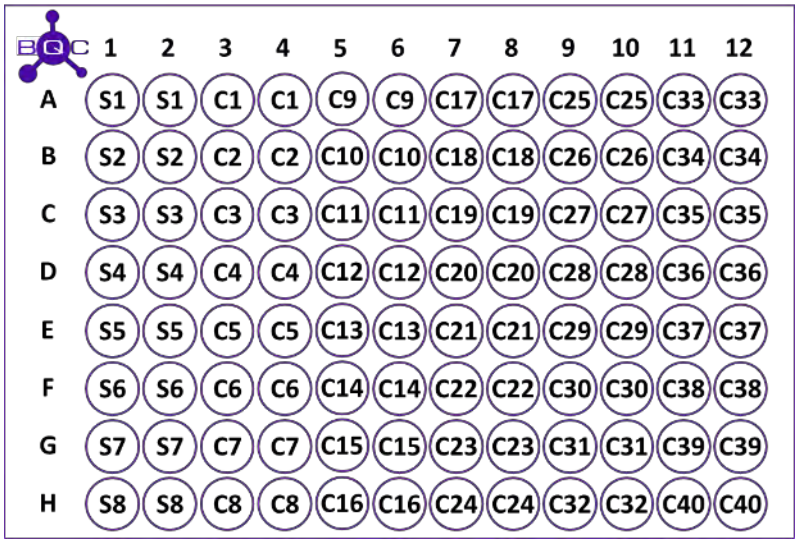
Table 3. *Microplate Standard Dilutions*

Sample	Standard [µL]	Diluent [µL]	Protein [µg]
S1(Blank)	---	200	---
S2	5	195	1.25
S3	10	190	2.5
S4	20	180	5
S5	40	160	10
S6	60	140	15
S7	80	120	20
S8	100	100	25

Assay Protocol

4. Add 250 µL of Bradford Reagent to each well and vortex using a microplate mixer.
5. Incubate at room temperature for at least 5 min. Samples should not be incubated longer than one hour at room temperature.
6. Measure the absorbance of these standards, blanks and unknown samples at 595 nm.

Plate set up



Attention

- This scheme is just a recommendation of how to perform the assay.
- For optimal results, it is recommended to run the standards and the samples for duplicate, but it is the user´s discretion to do so.

Data Analysis

1. If the spectrophotometer or microplate reader was not zeroed with the blank, then average the blank values and substract the average blank value from the standard and unknown sample values.

2. Create a standard curve by plotting A 595 nm (y-axis) vs. standard, µg (x-axis). Determine the unknown sample concentration using the standard curve.

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

4. Standard curve example for microplate assay procedure is shown in Figure 2.

5. Measure the absorbance of these standards, blanks and unknown samples at 595 nm.

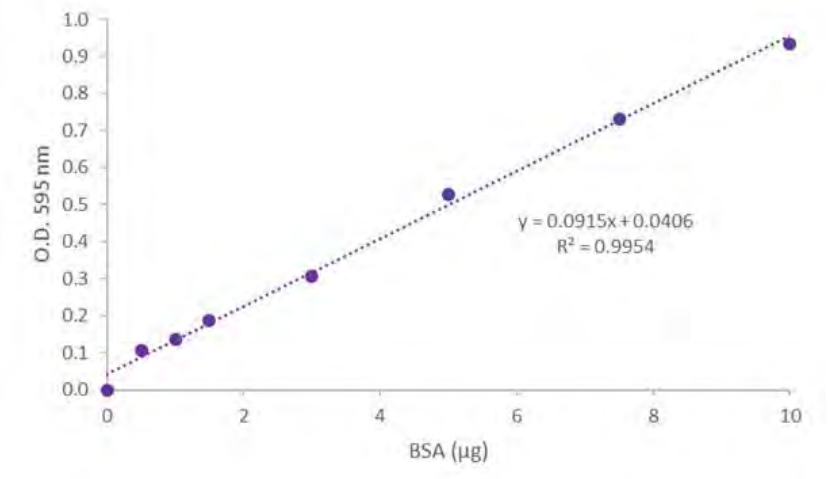


Figure 2. *Typical standard curve for Bradford assay*

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Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

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