

BCA Protein Quantification Assay Kit

KB-03-005

200/1000 test (96 well plate)

BOCKit

A brand of  **BioQuoChem**

Index

Introduction	Pag. 1
Materials	Pag. 2
Assay Principle	Pag. 3
Reagent Preparation	Pag. 8
Assay Protocol	Pag. 9
Data Analysis	Pag. 12
Warranties and Limitation of Liability	Pag. 13



All chemicals should be handled with care



➤ **This kit is for R&D use only**

Introduction

The BCA protein assay was first described in 1985 to determine protein concentration with the use of bicinchoninic acid (BCA). In common with the Lowry assay, the biuret reaction is the first step in the reaction that takes place in the BCA assay. In this reaction, protein reduces Cu^{2+} to Cu^{+} in an alkaline environment. In the second step, BCA reacts with Cu^{+} -ions to form a purple colored complex that has an absorbance at 562 nm. In Figure 1, the two-step reaction can be seen.

The absorbance increases linearly with increasing protein concentration over a broad working range (20-2000 $\mu\text{g}/\mu\text{L}$). Although the method consists of two reactions, only single reagent addition is required. However, due to the greater reactivity between the peptide bonds and copper at high temperatures, heating is required to increase absorbance in the presence of protein, improve sensitivity, lower protein-to-protein variability, and shorten incubation times.

BCA protein quantification kit is advantageous in that it does not interact with as many contaminants and buffer components as the Folin-Ciocalteu reagent, especially detergents. Components that interfere with the BCA protein quantification kit either lead to the reduction of Cu^{2+} (as DTT) or copper chelators (as EGTA). Generally, these

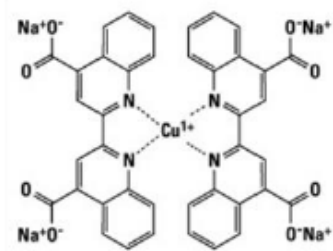
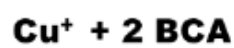
Introduction

are not critical components of buffers and can be easily removed or omitted prior to the assay.

Step 1.



Step 2.



Cu⁺ - BCA complex

Figure 1. The chemistry of the BCA method for the quantification of proteins consisting of a biuret reaction (step 1) and the formation of the complex between bicinchoninic acid (BCA) and monovalent copper ion (step 2). (Adaptation of Smith et al., 1985)

Materials

BQCKit BCA Protein Quantification Assay kit **KB03005-200 tests** contains:

Product	Quantity	Storage
Reagent A	1 bottle	RT
Reagent B	1 vial	RT
Protein Standard*	1 vial	4°C

BQCKit BCA Protein Quantification Assay kit **KB03005-1000 tests** contains:

Product	Quantity	Storage
Reagent A	2 bottles	RT
Reagent B	1 vial	RT
Protein Standard*	2 vials	4°C

*This reagent is stable for 10 days and is shipping at Room Temperature. Once received it is recommended to keep it at 4°C.

Assay Principle

The BCA method combines the biuret reaction with the colorimetric detection of the monovalent copper ion by bicinchoninic acid (BCA).

After the reduction of the divalent copper ion, Cu^+ reacts with BCA and the purple colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. The first reaction occurs at lower temperatures and is the result of copper and BCA interaction with aminoacid residues in the protein.

At elevated temperatures, the peptide bond is responsible for color development. Hence performing the assay at 37°C or 60°C versus room temperature increases the sensitivity and reduces the variation in the response of the assay as a function of protein composition. When possible, the assay should be incubated at 60°C since, after reaction is complete, the absorbance does not increase appreciably, whereas after cooling samples incubated at 37°C to room temperature, the blank continues to increase in absorbance at $\sim 2.3\%$ every 10 min.

The BCA assay has many advantages over other protein determination techniques due to there is less susceptibility to detergents, is easy to use and the color complex is stable.

Assay Principle

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

Substance	Compatible concentration
<i>Buffers</i>	
N-Acetylglucosamine (10 mM) in PBS, pH 7.2	100 mM
ACES, pH 7.8	25 mM
Bicine, pH 8.4	20 mM
Bis-Tris, pH 6.5	33 mM
CellLycticä B Reagent undiluted	Undiluted/no interference
Calcium chloride in TBS, pH 7.2	10 mM
CHES, pH 9.0	100 mM
Cobalt chloride in TBS, pH 7.2	0.8 mM
EPPS, pH 8.0	100 mM
Ferric chloride in TBS, pH 7.2	10 mM
HEPES	100 mM
MOPS, pH 7.2	100 mM
Nickel chloride in TBS	10 mM
PBS; Phosphate (0.1 M), NaCl (0.15 M), pH 7.2	Undiluted/no interference
PIPES, pH 6.8	100 mM
Sodium acetate, pH 4.8	200 mM
Sodium citrate, pH 4.8 or pH 6.4	200 mM
Tricine, pH 8.0	25 mM
Triethanolamine, pH 7.8	25 mM
Tris	250 mM
TBS; Tris (25 mM), NaCl (0.15 M), pH 7.6	Undiluted/no interference
Tris (25 mM), Glycine (1.92 M), SDS (0.1%), pH 8.3	Undiluted/no interference
Zinc chloride (10 mM) in TBS, pH 7.2	10 mM
<i>Buffer additives</i>	
Ammonium sulfate	1.5 M
Aprotinin	10 mg/L

Assay Principle

Cesium bicarbonate	100 mM
Glucose	10 mM
Glycerol	10 %
Guanidine HCl	4 M
Hydrochloric acid	100 mM
Imidazole	50 mM
Leupeptin	10 mg/l
PMSF	1 mM
Sodium azide	0.20%
Sodium bicarbonate	100 mM
Sodium chloride	1 M
Sodium hydroxide	100 mM
Sodium phosphate	25 mM
Sucrose	40 %
TLCK	0.1 mg/l
TPCK	0.1 mg/l
Sodium orthovanadate in PBS, pH 7.2	1 mM
Thimerosal	0.01 %
Urea	3M

Chelating agents

EDTA	10 mM
EGTA	Not Compatible
Sodium citrate	200 mM

Detergents

Brijä 35	5 %
Brij 52	1 %
CHAPSO	5 %
Deoxycholic acid	5 %
Nonidet P-40	5 %
Octyl β -glucoside	5 %
Octyl β -thioglucopyranoside	5 %
SDS	5 %

Assay Principle

Span 20	1 %
TRITON® X-100	5 %
TRITON® X-114	1 %
TRITON® X-305	1 %
TRITON® X-405	1 %
TWEEN® 20	5 %
TWEEN® 60	5 %
TWEEN® 80	5 %
Zwittergents	1 %

Reducing and thiol containing Reagents

Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
2-Mercaptoethanol	1 mM
Tributyl Phosphine	0.01 %

Solvents

Acetone	10 %
Acetonitrile	10 %
DMF	10 %
DMSO	10 %
Ethanol	10 %
Methanol	10 %

*The amount listed is the maximum amount of the material allowed in the protein sample without causing a noticeable interference.

Note: 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 interference exists.

Important: Reagents that chelate metal ions, change the pH of the assay, or reduce copper will interfere with the BCA assay.

Reagent Preparation

BCA Working Solution: Use the following formula to determine the total volume of Working Solution required:

$$\text{(standards + samples) x (replicates) x (volume of Working Solution per sample) = Total volume Working Solution Required}$$

50:1 (v/v) Reagent A/ Reagent B. Prepare fresh daily

Example: For 50 wells: 10 mL of BCA Working Solution are required. This is 9.8 mL of Reagent A and 0.2 mL of Reagent B.

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 BCA Protein Quantification Kit Micro assay (1.5 mL)

1. Prepare the calibrate in 1.5 mL tubes following the Table 2. For the diluent, use the same buffer as in the samples.
2. Pipette 75 μL of each standard or unknown sample solution into separate 1.5 mL tubes.

Table 2. *Microassay Standard Dilutions*

Sample	Standard [μL]	Diluent [μL]	Protein [μg]
S1 (Blank)	---	200	---
S2	4	196	15
S3	8	192	30
S4	12	188	45
S5	16	184	60
S6	20	180	75
S7	24	176	90
S8	28	172	105
S9	32	168	120

3. To each tube, add 1425 μL of freshly prepared BCA Working Solution.
4. Incubate the tubes 15 min at 60°C.
5. Cool all samples to room temperature.
6. Mix the samples, zero the spectrophotometer with the blank and measure the absorbance at 562 nm.

Assay Protocol

BQC BCA Protein Quantification Kit Microplate

1. Prepare the calibrate in 1.5 mL tubes following the Table 3. For the diluent use the same buffer as in the samples.
2. Pipette 10 μL of each standard and unknown sample replicate into a microplate well.
3. Add 200 μL of freshly prepared **BCA Working Solution** and immediately mix the microplate on plate mixer for 30 seconds.
4. Cover and incubate the microplate 15 min at 60°C.
5. Cool plate to room temperature.
6. Mix the samples, zero the spectrophotometer with the blank and measure the absorbance at 562 nm.

Table 3. Microplate Standard Dilutions

Sample	Standard [μL]	Diluent [μL]	Protein [μg]
S1 (Blank)	---	200	---
S2	4	196	2
S3	8	192	4
S4	12	188	6
S5	16	184	8
S6	20	180	10
S7	24	176	12
S8	28	172	14
S9	32	168	16

*The assay has a total linear protein range of 0-20 μg .

Assay Protocol

Plate set up

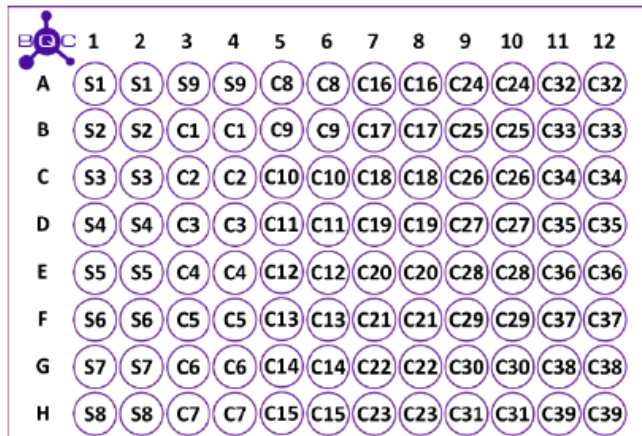


Figure 2. 96-well plate filling format

S1-S9 = Standards

C1-C39 = Samples

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 subtract the average blank value from the standard and unknown sample values.
2. Create a standard curve by plotting O.D. 562 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 3.
5. Measure the absorbance of these standards, blanks and unknown samples at 562 nm.

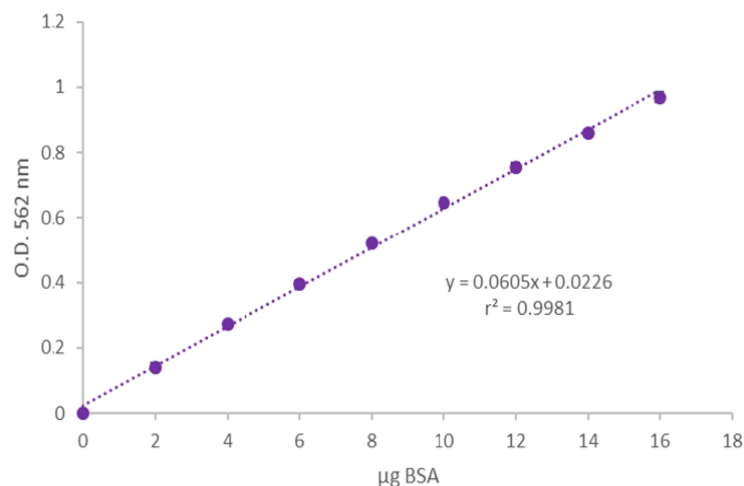


Figure 3. Typical standard curve using the microplate procedure

Warranties and Limitation of Liability

Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, down time, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by Bioquochem's gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by buyer for product.

Buyer's exclusive remedy and Bioquochem's sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

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

For further details, please refer to our website www.bqckit.com.

