LIBIOS
Dynamic Test Kits for R&D and Quality Control

BCA Protein Quantification Assay Kit

KB-03-005

BQCkit
A brand of Bio QuaChem

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All chemicals should be handled with

- 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 µg/µL). 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

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

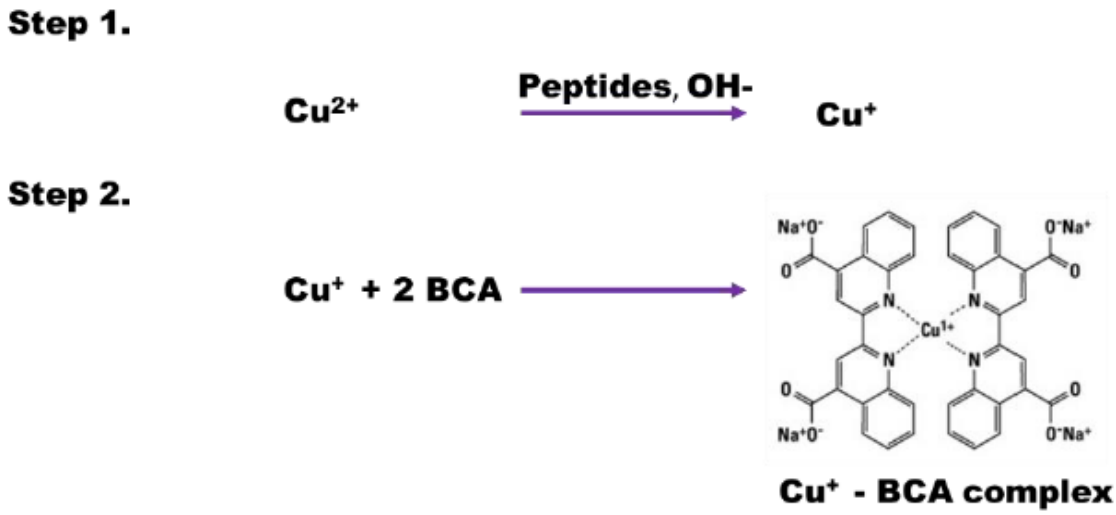


Figure 1. *The chemistry of the BCA method for the quantification of proteins consisting of a biuret reaction (step 1) and the formation of the complex between bicinchoninic acid (BCA) and monovalent copper ion (step 2). (Adaptation of Smith et al., 1985)*

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	4ºC

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	4ºC

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

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 37ºC or 60ºC 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 60ºC since, after reaction is complete, the absorbance does not increase appreciably, whereas after cooling samples incubated at 37ºC to room temperature, the blank continues to increase in absorbance at ~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
Buffers	
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
CelLyticä 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
Buffer additives	
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 -glucoside	5 %
Octyl -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 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. 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 or Working Solution required:

(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 60ºC.
5. Cool all samples to room temperature.
6. Mix the samples, zero the spectrophotometer with the blank and measure the absorbance at 562 nm.

BQC BCA Protein Quantification Kit Microplate

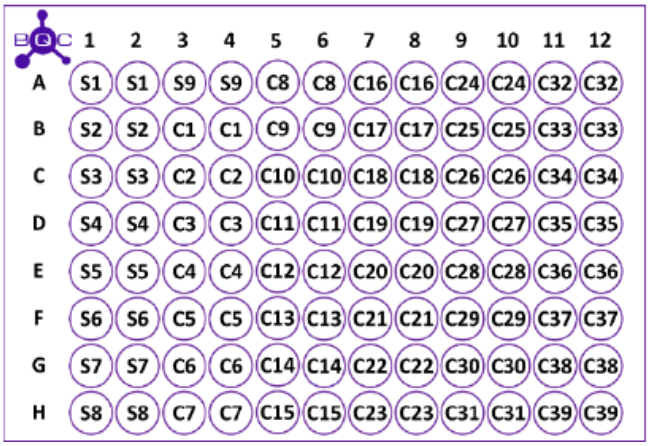
1. Prepare the calibrate in 1.5 mL tubes following the Table 3. For the diluent use the same buffer as in the samples.
2. Pipette 10 µL of each standard and unknown sample replicate into a microplate well.
3. Add 200 µL of freshly prepared BCA Working Solution and immediately mix the microplate on plate mixer for 30 seconds.
4. Cover and incubate the microplate 15 min at 60ºC.
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.

Plate set up



	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	C8	C8	C16	C16	C24	C24	C32	C32
B	S2	S2	C1	C1	C9	C9	C17	C17	C25	C25	C33	C33
C	S3	S3	C2	C2	C10	C10	C18	C18	C26	C26	C34	C34
D	S4	S4	C3	C3	C11	C11	C19	C19	C27	C27	C35	C35
E	S5	S5	C4	C4	C12	C12	C20	C20	C28	C28	C36	C36
F	S6	S6	C5	C5	C13	C13	C21	C21	C29	C29	C37	C37
G	S7	S7	C6	C6	C14	C14	C22	C22	C30	C30	C38	C38
H	S8	S8	C7	C7	C15	C15	C23	C23	C31	C31	C39	C39

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 substract 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.

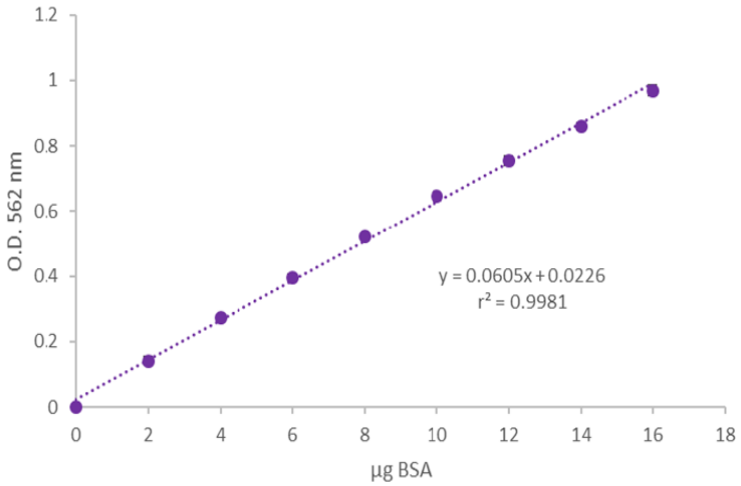


Figure 3. *Typical standard curve using the microplate procedure*

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Expiration date: 1 year from the date of delivery

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