BCA Protein Quantification kit

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BCA Protein Quantification kit
#D05077.200 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

#D11077
Version: 0117

Table of contents

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

This kit contains:

Designation	Item #	Quantity per kit	Form
BCA Protein Quantification Reagent A	D22077.200 dtn	1	Liquid
BCA Protein Quantification Reagent B	D20077.200 dtn	1	Liquid
BCA Protein Quantification Standard	D06077.1 ea	1	Liquid
Technical Booklet	D11077.1 ea	1	-

Each kit contains sufficient reagents for:

- 200 tests in 96-well plate. This allows for the construction of one standard curve in duplicate and the assay of 91 samples in duplicate (sample volume of 10 µL).

  or

- 28 tests in 1.5 mL tubes. This allows for the construction of one standard curve in duplicate and the assay of 5 samples in duplicate (sample volume of 75 µL).

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 incubations are done at 60°C. Alternative protocol at 37°C is possible but in this case after cooling samples to room temperature, the absorbance of blank continues to increase at ~2.3% every 10 minutes.

Higher temperatures (+37°C and +60°C) increase the sensitivity of the assay compared to room temperature.

Background

The protein quantification method using bicinchoninic acid (BCA) ***[1]*** has been described for the first time in 1985.

The method is based on the reaction between peptide bonds and four particular amino acids (cysteine, cystine, tryptophan and tyrosine) ***[2]*** with copper. Consisting in two reactions, only one reagent addition is required.

The quantification of proteins by BCA is advantageous because there is less interferences with contaminants or buffer components compared to the Folin-Ciocalteu reagent and specially with detergents.

Principle of the assay

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

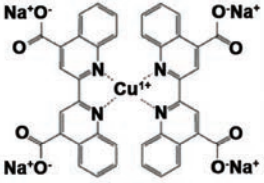
After the reduction of the divalent copper ion by peptide bonds and four particular amino acids, one molecule of Cu^{+} is chelated by two molecules of BCA to form a purple compound that absorbs between 540-570 nm.

Step 1

Cu^{2+} —(Peptides, OH-)→ Cu^{+}

Step 2

$Cu^{+} + 2\ BCA \rightarrow$



Cu^{+} - BCA complex

The absorbance increases linearly with protein concentration over a broad working range ***[2]*** (20-2000 µg/mL).

Materials and equipment required

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

For the microplate assay:

- Precision pipettes & multichannel pipettes (300 µL)
- Disposable tips
- Polypropylene microtubes (1.5 mL) and tubes
- 96-well plate
- Orbital microplate shaker
- Microplate reader (540 to 570 nm filter)

For the microtube assay:

- Precision micropipettes (50-5000 µL)
- Disposable tips
- Polypropylene microtubes (1.5 mL) and tubes
- Spectrophotometer (540 to 570 nm filter)

Sample collection and preparation

No particular attention for the collection and preparation of the samples excepted for sample containing reducing agent of Cu^{2+} like DTT but also chelating agent of copper like EGTA. In this case, remove on desalting column or dilute the interference.

Strong acid or alkaline buffers may also affect the reaction.

Find hereafter a table of reagents compatible with the kit protein assay 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.

Substance	Compatible concentration
Buffers and additives	
ACES pH 7.8	25 mM
Ammonium sulfate	1.5 M
Aprotinin	10 mg/L
Bicine pH 8.4	20 mM
Bis-Tris pH 6.5	33 mM
Calcium chloride in TBS pH 7.2	10 mM
CelLyticä B Reagent	Undiluted
Cesium bicarbonate	100 mM
CHES pH 9.0	100 mM
Cobalt chloride in TBS pH 7.2	0.8 M
EPPS pH 8.0	100 mM
Ferric chloride in TBS pH 7.2	10 mM
Guanidine HCl	4 M
Glucose	10 mM
Glycerol	10 %
HEPES	100 mM
Hydrochloric acid	100 mM
Imidazole	50 mM
Leupeptin	10 mg/L
MOPS pH 7.2	100 mM
N-Acetylglucosamine 10 mM pH 7.2 in PBS	10 mM
Nickel chloride in TBS	10 mM
PBS pH 7.2	Phosphate 0.1 M-NaCl 0.15 M
PIPES pH 6.8	100 mM
PMSF	1 mM
Sodium acetate pH 4.8	200 mM
Sodium azide	0.20%
Sodium bicarbonate	100 mM

Substance	Compatible concentration
Buffers and additives (continue)	
Sodium chloride	1 M
Sodium citrate pH 4.8 or pH 6.4	200 mM
Sodium hydroxide	100 mM
Sodium orthovanadate in PBS pH 7.2	1 mM
Sodium phosphate	25 mM
Sucrose	40 %
TBS pH 7.6	Tris 25 mM-NaCl 0.15 M
TLCK	0.1 mg/L
Thimerosal	0.01 %
Tricine pH 8.0	25 mM
Triethanolamine pH 7.8	25 mM
Tris	250 mM
Tris-Glycine-SDS pH 8.3	Tris 25 mM-Glycine 1.92 M-SDS 0.1%
TPCK	0.1 mg/L
Urea	3 M
Zinc chloride in TBS pH 7.2	10 mM

Substance	Compatible concentration
Chealating agents	
EDTA	10 mM
Sodium citrate	200 mM

Substance	Compatible concentration
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 %
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 %

Substance	Compatible concentration
Reducing and thiol containing reagents	
Dithioerythritol (DTE)	1 mM
Dithiothreitol (DTT)	1 mM
2-Mercaptoethanol	1 mM
Tributyl Phosphine	0.01 %

Substance	Compatible concentration
Solvents	
Acetone	10 %
Acetonitrile	10 %
DMF	10 %
DMSO	10 %
Ethanol	10 %
Methanol	10 %

Reagent preparation

BCA Working Solution

The BCA Working Solution is a mix of the Reagent A and the Reagent B at 50:1.

Working Solution = 50:1 (v/v) Reagent A / Reagent B

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

(standards + samples) x (replicates) x (volume of Working Solution per sample*)

* *volume of Working Solution per sample is 1425 µl for the Microtube procedure*
* *volume of Working Solution per sample is 200 µL for the Microplate procedure*

Stability at 4°C: use within the day.

***Example**: If you need 50 mL of BCA Working Solution, mix 50 mL of Reagent A and 1 mL of Reagent B.*

BCA protein Quantification Standard

Prepare the standards in 1.5 mL microtubes following the table hereafter:

The diluent has to be the same buffer as in the samples

Sample	Standard (µL)	Diluent (µL)	Protein (µg/mL)
S9	32	168	1600
S8	28	172	1400
S7	24	176	1200
S6	20	180	1000
S5	16	184	800
S4	12	188	600
S3	8	192	400
S2	4	196	200
S1(Blank)	-	200	0

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	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

S1-S9 : Standards C1-C39: 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 procedure (1.5 mL tube)		Microplate procedure (96-well plate)	
	Standard	Samples	Standard well	Sample well
Standard	75	-	10	-
Sample	-	75	-	10
BCA Working Solution	1425		200	
	-		Mix immediately the microplate on an orbital shaker at 300 rpm	
Incubate at 60°C(*) during 15 min in the dark				
Cool the plate or the tubes at room temperature during 5 min				
Mix 1 min at 300 rpm				
Read the plate between 540 nm and 570 nm				

Optional: you may zero the spectrophotometer with the Blank (S1) first.
(*) Incubation 30 min at +37°C is possible but after cooling standards/samples at room temperature, the Blank absorbance continues to increase at ~2.3% every 10 minutes.

NB : 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. Increase the incubation time if higher 0.D values are desired.

For both procedures, it is also possible to increase the incubation time to detect lower protein concentrations.

Please ensure that both samples and standards are assayed the same way.

Data analysis

- If the spectrophotometer or the microplate reader were not zeroed with the Blank, subtract the average blank value to the other absorbance readings.

- Create a standard curve by plotting O.D. (y-axis) vs standard, µg/mL (x-axis). Determine the unknown sample concentration using the standard curve. Samples higher than 2000 µg/mL must be diluted and re-assayed. For samples below 20 µg/mL redo the assay increasing time and/or temperature (max. +60°C).

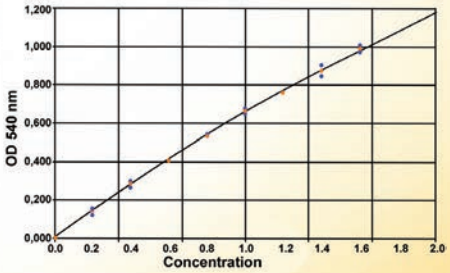
Typical results

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 540 nm. A 4-parameter logistic fitting was used to determine the concentrations.

Standard	[Standard] µg / mL	Absorbance A.U.
S9	1600	0.989
S8	1400	0.873
S7	1200	0.761
S6	1000	0.663
S5	800	0.538
S4	600	0.406
S3	400	0.285
S2	200	0.137
S1	0	0.000

Typical BCA standard curve



Troubleshooting

> **No colour:**
- Sample may contain a copper chelating agent .
  Solution: Dialyze, desalt or dilute sample

> **Blank absorbance is OK, but standards and samples show less colour than expected**:
- Strong acid or alkaline buffer alter working reagent pH.
  Solution: Dialyze, desalt or dilute sample

- Colour measured at the wrong wavelength.
  Solution: make sure the reading is performed between 540-570 nm

> **Colour of samples appears darker than expected**:
- Protein concentration is too high.
  Solution: dilute sample

- Sample contains lipids or lipoproteins
  Solution: Add 2% SDS to the sample to remove lipid interference

> **All tubes (including Blank) are dark purple**:
- Buffer contains reducing agent, thiol or biogenic amines (catecholamines).
  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).

Bibliography

1. Smith PK., Krohn RI., Hermanson GT. *et al.*
Measurement of protein using bicinchoninic acid.
Anal. Biochem.150, 76–85 (1985).

2. Wiechelman KJ., Braun RD., Fitzpatrick JD.
Investigation of the bicinchoninic acid protein assay:
Identification of the groups responsible for color formation.
Anal Biochem 175:231-237 (1988).

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