Protocol Booklet

Product Code(s)	HB6653
Product Name	Mini BCA Protein Assay Kit
Purpose	Measurement of protein concentration in solution.

Please note: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Contents

Product Overview

Simple, rapid, detergent tolerant (up to 5%) kit for determining the concentration of proteins in solution. This kit is optimized to measure protein concentrations from 0.5 to 200 µg/ml.

Key features of the mini-BCA Protein Assay Kit:

- **Detergent compatible** - compatible with detergent concentrations up to 5%
- **Wide assay range** - can measure protein concentrations from 0.5 to 200 µg/ml
- **Stability** - kit is stable at room temperature

Components & Storage

This kit contains:

- Mini BCA Assay Reagent A
- Mini BCA Assay Reagent B
- Mini BCA Assay Reagent C
- Recombinant albumin protein standard (200µg/ml)

Note: Store all components at room temperature.

This kit additionally requires:

- 96-well microplates and microplate reader (for microplate assays)
- Test tubes, cuvettes and spectrophotometer (for tube assays)
- Incubator or water bath for incubations

Protocol

Preparing reagents and general advice

Before using the kit for the first time add 10ml of buffer to the tube of lyophilised standard to create a 200µg/ml solution. This can then be used to generate the standards required for a standard curve or subdivided and snap frozen for future use. Create a dilution series of standards using the table below (this will generate enough to run the standards in triplicate), these can also be snap frozen and reused. For best accuracy use the same buffer as your proteins of interest. The assay can either be carried out in microplate or tube format.

Standard	Concentration (mg/ml)	Volume buffer	Volume Stock	Final volume[a] (µl)	Remaining volume[b] (µl)
A	200µg/ml	0µl	1000µl of 200µg/ml stock	1000µl	700µl
B	40µg/ml	1700µl	300µl of 200 µg/ml stock	1500µl	750µl
C	20µg/ml	750µl	750µl of 40µg/ml stock	1500µl	750µl
D	10µg/ml	750µl	750µl of 20µg/ml stock	1500µl	750µl
E	5µg/ml	750µl	750µl of 10µg/ml stock	1500µl	800µl
F	2.5µg/ml	500µl	500µl of 5µg/ml stock	1000µl	1000µl
G	1µg/ml	800µl	200µl of 5µg/ml stock	1000µl	500µl
H	0.5µg/ml	500µl	500µl of 1µg/ml stock	1000µl	1000µl
I	0µg/ml	1000µl	0µl	1000µl	1000µl

a. Volume when stock and buffer mixed together
b. Volume after stock has been taken to make subsequent dilutions.

Microplate Protocol

1. Add 150µl of each standard to a microplate well alongside the unknown samples. Ideally this should be carried out in triplicate.
2. Prepare the working reagent by mixing 1 volume of reagent C with 25 volumes reagent B. Then add 26 volumes reagent A to the C/B mixture. For easy calculation add 3µl reagent C, 75µl reagent B and 78µl reagent A per well.
3. Add 150µl of working reagent to each well and incubate for 2 hours at 37°C
4. Let the plate cool to room temperature for 1-2 minutes.
5. Measure absorbance at 562nm using a microplate reader. This should be ideally carried out within 40 minutes of the start of the assay to maximise accuracy.
6. Subtract the absorbance of the 0mg/ml samples from all measurements then construct a standard curve using the sample data.
7. Use the standard curve to calculate the protein concentration of the unknown samples.

Tube Protocol

1. Add 150µl of each standard to a microplate well alongside the unknown samples. Ideally this should be carried out in triplicate.
2. Prepare the working reagent by mixing 1 volume of reagent C with 25 volumes reagent B. Then add 26 volumes reagent A to the C/B mixture. For easy calculation add 3µl reagent C, 75µl reagent B and 78µl reagent A per well.
3. Add 150µl of working reagent to each well and incubate for 2 hours at 37°C
4. Let the plate cool to room temperature for 1-2 minutes.
5. Measure absorbance at 562nm using a microplate reader. This should be ideally carried out within 40 minutes of the start of the assay to maximise accuracy.
6. Subtract the absorbance of the 0mg/ml samples from all measurements then construct a standard curve using the sample data.
7. Use the standard curve to calculate the protein concentration of the unknown samples.

Guidelines, precautions, troubleshooting

Please follow the below table to resolve any problems encountered when using this kit. For any problems not listed or for any further advice please contact our technical support team at technicalhelp@hellobio.com.

Problem	Potential Cause
No signal in any tubes	The sample contains a copper chelating agent which interferes with the mechanism of the assay. If possible try to remove the chelator through dialysis / desalting or try increasing the concentration of reagent C.
Sample absorbance is out of range	If the absorbance of the unknown samples is out of range for the standard curve then try doing a 1:10 dilution and re-measuring absorbance. Remember to account for this dilution when working out the original protein concentration.
No equipment within lab able to measure at 562nm	It is possible to measure absorbance between 540 and 590nm however this may effect the sensitivity of the assay.
All tubes (including blank) are dark purple	The buffer may contain a reducing agent, thiol or biogenic amine which disrupt the mechanism behind the assay. Try dialysing / desalting the sample or dilute the sample until the interference ceases.
Standards and samples do not develop enough of a color change	Strongly acidic or alkaline buffers will effect the pH of the working reagent which will interfere with the assay. Try diluting the samples by using dialysis / buffer exchange to remove the strongly basic or acidic elements of the buffer.

Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the assay kit.

Contact

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