Protocol Booklet

Product Code(s)	HB7109
Product Name	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 kit for determining the concentration of proteins in solution. This kit is optimized to measure protein concentrations from 0.02 to 2mg/mL.

Key features of the BCA Protein Assay Kit:

- **Dilution free** - contains 11 pre-diluted recombinant albumin standards for easy standard curve construction.
- **Detergent compatible** - compatible with detergent concentrations up to 5%
- **Quick** - 30 minute incubation time means total assay time of around 45 minutes.
- **Wide assay range** - can measure protein concentrations from 0.02 to 2 mg/ml
- **Stability** - kit is stable at room temperature

Components & Storage

This kit contains:

- BCA Assay Reagent A
- BCA Assay Reagent B
- Recombinant albumin protein standards x 11 (0 – 2mg/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 2ml of buffer to each tube of lyophilised standard. For best accuracy use the same buffer as your proteins of interest. The assay can either be carried out in microplate or tube format. This will give the following standards:

Standard	Concentration (mg/ml)
A	2
B	1.5
C	1
D	0.75
E	0.5
F	0.25
G	0.125
H	0.025
I	0.005
J	0.001
K	0

Microplate Protocol

1. Add 25µl 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 50 parts reagent A with 1 part reagent B. For easy calculation add 200µl reagent A and 4µl reagent B per well.
3. Add 200µl of working reagent to each well and incubate for 30 minutes 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 100µl of each standard to labelled test-tubes alongside the unknown samples. Ideally this should be carried out in triplicate.
2. Prepare the working reagent by mixing 50 parts reagent A with 1 part reagent B. For easy calculation add 2ml reagent A and 40µl reagent B per tube.
3. Add 2ml of working reagent to each tube and mix thoroughly.
4. Incubate for 30 minutes at 37°C. Alternatively the incubation can be carried out for 60 minutes at room temperature.
5. Cool all tubes to room temperature for 1-2 minutes.
6. Measure absorbance at 562nm using a spectrophotometer ensuring that all measurements are made within 10 minutes. Prior to taking the measurements ensure the spectrophotometer is blanked using a cuvette filled with dH2O.
7. Subtract the absorbance of the 0mg/ml samples from all measurements then construct a standard curve using the sample data.
8. 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 B.
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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