Amplite™ Colorimetric BCA Protein Quantitation Assay Kit

Catalog number: 11115, 11116
Unit size: 1000 Tests, 5000 Tests

Component	Storage	Amount (Cat No. 11115)	Amount (Cat No. 11116)
Component A: BCA Solution A	Refrigerated (2-8 °C), Minimize light exposure	50 mL	250 mL
Component B: BCA Solution B	Refrigerated (2-8 °C), Minimize light exposure	1 mL	5 mL
Component C: BSA Standard (2 mg/mL)	Freeze (< -15 °C), Minimize light exposure	1 mL	5 mL

OVERVIEW

The traditional BCA protein assay is widely used for quantifying proteins. However, it is slow and tedious, e.g., you must either heat the BCA reaction at 37 °C for 30 minutes or wait for two hours at room temperature. Amplite™ Colorimetric BCA Protein Quantitation Assay Kit is a two-component and detergent-compatible assay to determine total protein concentrations. The assay is based on the same copper-chelating reaction and provides comparable accuracy to the traditional BCA protein assay that is either run at high temperature or with longer incubation time. The protein signal is monitored around 560 nm and the assay is completed within 30 minutes. It is convenient, rapid, and robust without high temperature required. Amplite™ Colorimetric BCA Protein Quantitation Assay Kit can be performed in a convenient 96-well microtiter-plate format and easily adapted to automation with no separation steps required.

AT A GLANCE

Protocol summary

1. Prepare BCA working solution (50 µL)
2. Add BSA standards or test samples (50 µL)
3. Incubate at room temperature for 20 - 60 minutes
4. Read absorbance at 562 nm (in the range of 540-590 nm)

Important

Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	562 nm
Recommended plate	Clear bottom

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11115

BSA Standard

Add 80 µL of 2 mg/mL BSA Standard (Component C) to 320 µL of PBS (not provided) to generate 400 µg/mL BSA standard solution (BS1). Then use 1:2 serial dilutions in PBS to get serially diluted BSA standards (BS2 - BS7). Note: It is necessary to create a standard curve during each assay.

PREPARATION OF WORKING SOLUTION

BCA working solution

1. Prepare the amount of BCA working solution needed by mixing 50 parts of BCA Solution A (Component A) with 1 part of BCA Solution B (Component B) (50:1, v/v ratio of Solution A: B).
2. Mix well until the BCA working solution is a uniform, light green color.

Note 1 mL BCA working solution is enough for 20 tests.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of BSA standards and test samples in a clear bottom 96-well microplate. BS= BSA Standards (BS1 - BS7, 400 to 6.25 µg/mL); BL=Blank Control; TS=Test Samples

BS1	BS1	TS	TS
BS2	BS2	...	...
BS3	BS3		
BS4	BS4		
BS5	BS5		
BS6	BS6		
BS7	BS7		
BL	BL		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
BS1-BS7	50 µL	Serial dilutions (400-6.25 µg/mL)
BL	50 µL	PBS
TS	50 µL	Test samples

1. Prepare BSA standards (BS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2.
2. Add 50 µL of BCA working solution to each well of BSA standard, blank control, and test samples to make the total assay volume of 100 µL/well.
3. Incubate the reaction at room temperature for 20 to 60 minutes.
4. Monitor the absorbance with an absorbance microplate reader at OD 562 nm (in the range of 540 to 590 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

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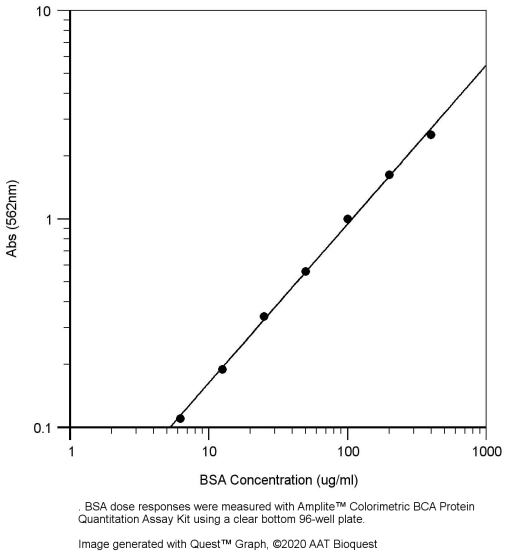

Figure 1. . BSA dose responses were measured with Amplite™ Colorimetric BCA Protein Quantitation Assay Kit using a clear bottom 96-well plate.

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