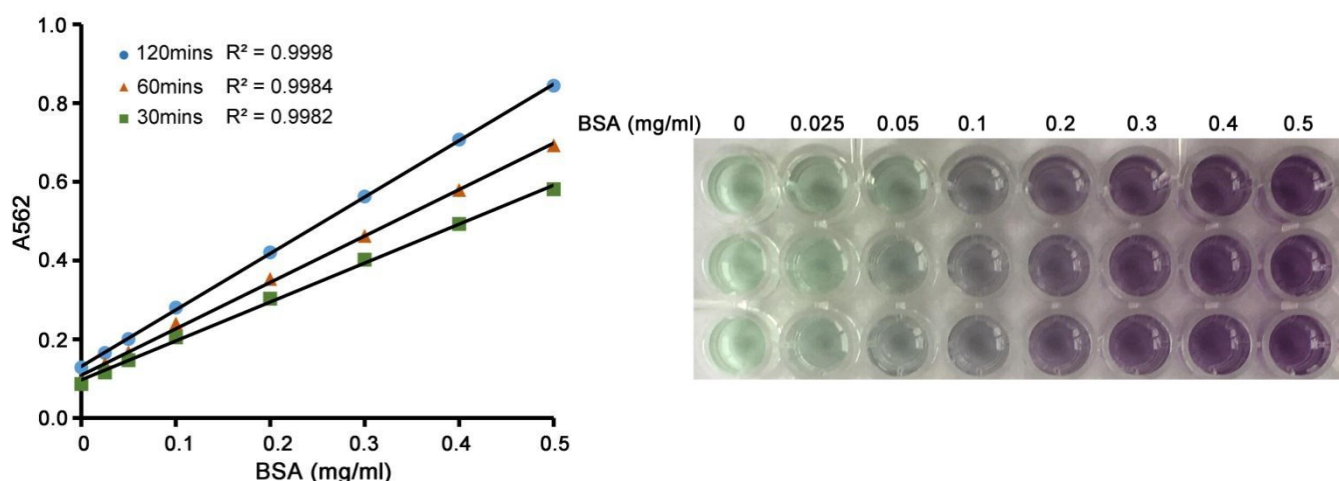


## Enhanced BCA Protein Assay Kit

Cat. No.	Product Name	Pack Size
P0009	Enhanced BCA Protein Assay Kit	5000T

### Description:

- Beyotime's Enhanced BCA Protein Assay Kit is an upgraded formulation based on the Bicinchoninic Acid (BCA) Protein Assay method which is one of the two most commonly used methods for determining protein concentrations. This product provides a simple, sensitive, and highly stable method for quantifying total protein in samples with high compatibility.
- Compared with the BCA Protein Assay Kit produced by Beyotime (P0011/P0012/P0012S), this kit has higher sensitivity, with the lower detection limit down to 10 $\mu$ g/ml and a minimum detection protein amount of 0.2 $\mu$ g. The microplate protocol requires a sample volume of 1-20 $\mu$ l.
- Compared with the BCA Protein Assay Kit produced by Beyotime (P0011/P0012/P0012S), the color development of this product is faster. It takes less time for color formation.
- This kit has a good linearity in the concentration range of 20-1000 $\mu$ g/ml. Please refer to Figure 1 for the standard curves obtained with this kit.



**Figure 1.** Standard curves of BSA obtained with Beyotime's Enhanced BCA Protein Assay Kit (P0009). The picture on the left shows the standard curves obtained after incubation at 37 $^{\circ}$ C for different time courses as indicated. The picture on the right shows the actual color formation after incubation at 37 $^{\circ}$ C for 60 minutes. This figure is for reference only, which may vary due to different experimental conditions.

- BCA Protein Assay is not affected by the chemical substances in most samples. It is compatible with 5% of SDS, Triton X-100, Tween 20, 60, or 80 in samples. However, this assay can be interfered by chelators and higher concentrations of reductants. EGTA, EDTA higher than 10mM, DTT higher than 1mM, and  $\beta$ -Mercaptoethanol higher than 0.01% should be avoided from the assay. When the BCA Protein Assay method is not suitable for a sample, we recommend using the Bradford Protein Assay Kit (Beyotime, P0006).
- The detailed compatibility of Beyotime's Enhanced BCA Protein Assay Kit with various substances in samples is the same as that of the BCA Protein Assay Kit. Please check the following webpage for detailed information: <http://www.beyotime.com/CompatibilityChartForBCAKit.pdf>.
- This kit is sufficient for 500 assays, respectively, when the 96-well plate is used for the assay as described in the protocol.

### Packing List:

Item	Component	Quantity
P0009-1	BCA Reagent A	500ml $\times$ 2

P0009-2	BCA Reagent B	30ml
P0009-3	BSA Standard	30mg×2
P0009-4	BSA Preparation Solution	5ml
Manual	—	1 copy

## Storage Conditions:

Store at room temperature upon receipt. The BSA standard solution after preparation should be aliquoted and stored at -20°C.

## Precautions:

- A 96-well plate and a microplate reader that can measure the absorbance at 540-595nm (optimum at 562nm) are required. A spectrophotometer can also be used, but the assay volume should be adjusted based on the volume of the cuvette. When using a spectrophotometer, the number of samples that can be measured using this kit may be significantly reduced.
- If the sample dilution buffer or sample lysate itself generates a high background, please try Beyotime's Bradford Protein Assay Kit (P0006).
- In order to speed up the determination of protein concentration by BCA method, heat the reaction appropriately in a microwave oven, but do not overheat.
- This method is compatible with EDTA less than 10mM, but not EGTA. When the BCA method is not applicable, Beyotime's Bradford Protein Assay Kit (P0006) can be attempted.
- This product is for R&D only. Not for drug, household, or other uses.
- For your safety and health, please wear a lab coat and disposable gloves during the operation.

## Instructions for Use:

### 1. Preparation of BSA Standards

- a. Add 1.2ml of BSA Preparation Solution into one tube of BSA Standard (30mg BSA) provided in this kit to make a 25mg/ml BSA standard solution after fully dissolving. Keep an appropriate amount for immediate use. Aliquot and store the rest at -20°C for future use.
- b. Dilute an appropriate amount of 25mg/ml BSA standard solution to a final concentration of 0.5mg/ml (e.g., mix 20µl of 25mg/ml BSA standard solution with 980µl of diluent). The standard should be diluted with the buffer used for sample preparation, or with the 0.9% NaCl or PBS for simplicity. The diluted 0.5mg/ml BSA standard can be stored at -20°C for long term storage.

### 2. Preparation of BCA Working Solution

According to the number of assays, prepare an appropriate amount of BCA Working Solution by mixing BCA reagent A and BCA reagent B at a ratio of 50:1 (e.g., mix 5ml of BCA reagent A with 100µl of BCA reagent B) and mix well. The BCA Working Solution is stable for 24 hours at room temperature.

### 3. Determination of Protein Concentration

- a. Pipette 0, 1, 2, 4, 8, 12, 16, and 20µl of the 0.5mg/ml BSA standard solution to the standard wells of the 96-well plate, and add the sample preparation buffer to a final volume of 20µl per well, which is equivalent to 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml, respectively.
- b. Add an appropriate volume of sample to the sample wells of the 96-well plate. If the sample is less than 20µl, add the sample preparation buffer to a final volume of 20µl per well. Record the sample volume used.
- c. Add 200µl of BCA Working Solution to each well and incubate at 37°C for 20-30 minutes.

*Note: The reactions can also be incubated at room temperature for 2 hours, or at 60°C for 30 minutes. The color intensity of the reactions will continue to increase over time, and the color development will be accelerated by the increase of temperature. If the protein concentration of a sample is low, we recommend incubating at a higher temperature or extending the incubation time appropriately.*

- d. Measure the absorbance at 562nm or other wavelength between 540-595nm with a microplate reader.
- e. Calculate the protein concentration of the sample based on the standard curve and the sample volume used.

## FAQ:

- 1. When measuring the standard curve, it was found that the absorbance or color intensity did not change significantly with the increase of standard concentrations.**

The possible reason is that the sample preparation buffer contains substances that seriously interfere with the BCA Protein Assay. For a detailed information about the substances that cause interference with the BCA Protein Assay, please refer to our website at: <http://www.beyotime.com/Compatibility Chart For BCA Kit.pdf>

- 2. Do I need to make a standard curve for every measurement?**

It is recommended to make a standard curve for every measurement, because the color intensity of BCA reactions will increase over time and the color development is related to the temperature. Unless the time and temperature of the assay can be precisely controlled, a standard curve should be plotted for every measurement when accurate quantification is desired.

## Related Products:

Cat. No.	Product Name	Pack Size
P0006	Bradford Protein Assay Kit	1000T
P0006C	Detergent Compatible Bradford Protein Assay Kit	800T
P0007	BSA Standard (5mg/ml)	1ml
P0009	Enhanced BCA Protein Assay Kit	5000T
P0010	Enhanced BCA Protein Assay Kit	500T
P0010S	Enhanced BCA Protein Assay Kit	200T
P0011	BCA Protein Assay Kit	5000T
P0012	BCA Protein Assay Kit	500T
P0012S	BCA Protein Assay Kit	200T

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