# BCA protein concentration determination kit

**Item No. :** PC0020

**Specification:** 50T (500 microholes)

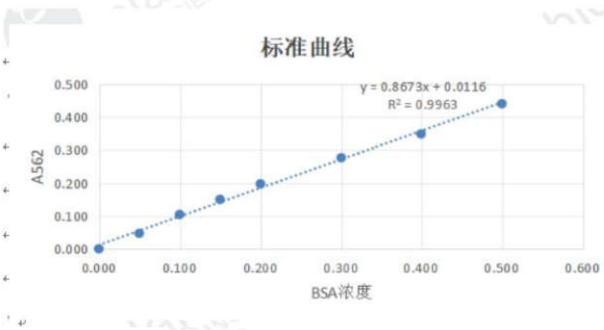
**Shelf life:** 2-8℃ storage, valid for 12 months.

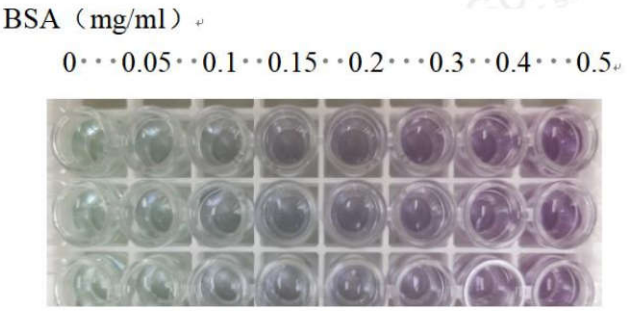
**Product content:**

| Ingredients | Packaging (500 microholes) | Save |
|---|---|---|
| BCA reagent | 100mL | Room temperature |
| Cu reagent | 3mL | Room temperature |
| PBS diluent | 30mL | Room temperature |
| BSA protein Standard (5mg/mL BSA) | 1mL | - 20℃ |

**Product introduction:**

Under alkaline condition, the protein will reduce $Cu^{2+}$ to $Cu^{+}$, $Cu^{+}$ and BCA reagent to form purple blue complex, determination of its absorption value at 562nm, and compared with the standard curve, you can calculate the concentration of the protein to be measured. The commonly used concentration of descaling agent SDS, Triton X-100, Tween does not affect the detection results, but is affected by chelating agents (EDTA, EGTA), reducing agents (DTT, mercaptoethanol) and lipids. In the experiment, if the background value of the sample diluent or lysate itself is found to be high, the Bradford protein concentration determination kit can be used.




In Figure 1, the left figure is the standard curve of detection by microplate method. The horizontal axis is the different concentration gradients of BSA, and the vertical axis is the absorption value at the corresponding 562nm. The figure on the right shows the actual color rendering effect of the standard product with different concentration gradients (incubation time 30min).

**Note: The data in the figure is for reference only, and the actual test results shall prevail.**

**Operation instructions (for reference only) :**

First, microporous enzyme labeling method

1. Preparation of working liquid: According to the number of standard products and samples, according to 50 volumes of BCA reagent and 1 volume of Cu reagent (50:1) preparation of BCA working liquid, fully mixed (there may be turbidity when mixing, but it will disappear after mixing).

The BCA working liquid is stable within 24 hours at room temperature.

2. Dilute standard: Take 10 microliters of BSA standard and dilute it with PBS to 100 microliters (the sample can generally be diluted with PBS), so that the final concentration is 0.5mg/mL. Add 0, 2, 4, 6, 8, 12, 16, 20 μl to the protein standard well of the 96-well plate, and add PBS to make up to 20μL, equivalent to the standard concentration of 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5mg/ml, respectively.

3. Dilute the sample appropriately (it is best to do several gradients, such as 2x, 4x, 8x dilution), and add 20 μL to the sample hole of the 96-well plate. Due to the large error of the pipette when taking a small amount of sample, the point in front of the standard line may not be very accurate, so as far as possible, let the sample point fall 1/2 behind the standard line.

4. Add 200 microliters of B CA working liquid to each hole and place at 37 ° C for 15-30 minutes. A562nm was measured with an enzyme marker, and the protein concentration was calculated according to the standard curve. When incubating in a warm box, care should be taken to prevent water evaporation from affecting the test results.

Second,. spectrophotometer method

If there is no enzyme label instrument, can be used spectrophotometer in the centrifuge tube mixed after adding the colorimetric dish to compare the color.

The steps are as follows :

1. Preparation of working liquid: according to the standard product and the number of samples, according to 50 volumes of BCA reagent plus 1 volume of Cu reagent (50:1) preparation of BCA working liquid, fully mixed (there may be turbidity when mixing, but it will disappear after mixing). The BCA working liquid is stable at room temperature for 24h.

2. Dilution standard: Take 100 microliters of BSA standard and dilute it to 1mL with PBS (the sample can generally be diluted with PBS), so that the final concentration is 0.5mg/mL.

3. Take eight (or more) 5mL centrifuge tubes, mark them, and add the reagent according to the following table. The corresponding standard concentrations are 0, 0.1, 0.2, 0.3, 0.4 and 0.5mg/mL respectively.

| Centrifuge tube number | 1 | 2 | 3 | 4 | 5 | 6 | 7 (Sample tube 1) | 8 (sample tube 2) | 9 (sample tube 3) |
|---|---|---|---|---|---|---|---|---|---|
| Standard protein BSA | 0 | 40μL | 80μL | 120μL | 160μL | 200μL | 200μL properly diluted sample 1 | 200μL properly diluted sample 2 | |
| PBS | 200μL | 160μL | 120μL | 80μL | 40μL | 0 | 0 | 0 | 0 |
| BCA Working fluid | 2mL | 2mL | 2mL | 2mL | 2mL | 2mL | 2mL | 2mL | 2mL |

4 Set aside at 37C for 15-30min. Use a spectrophotometer to measure the light absorption value at 562nm and calculate the protein concentration according to the standard curve.

**Points to note:**

1. When not used for a long time, Cu reagent and PBS diluent can be stored at 2-8℃, and should be discarded if bacterial contamination is found. When the BCA reagent is crystallized and precipitated at low temperature, it can be incubated at 37℃ to completely dissolve, without affecting the use.

2. If the sample contains more interfering substances, please use the Bradford protein concentration determination kit.

3. For your safety and health, please wear a lab coat and disposable gloves.

**Related products:**

*PC0001 BSA standard (5mg/mL)*
*PC0021 BCA reagent*
*PC0030 Lowry assay kit for protein concentration*
*PC0010 Bradford method protein concentration determination kit*
*R0010 High efficiency RIPA tissue/cell fast lysate*
*R0050 nuclear protein extraction kit*
*P1200 SDS-PAGE gel preparation kit*
*T1070 5 x TRIS-Glycine electrophoretic buffer*
*PR1600 prestain with low molecular weight protein Marker*
*P1015 4× Protein Loading buffer (DTT included)*
*D1060 10 x electrophoresis transfer buffer*
*PE0010 ECL Plus Fluorescence Detection Reagent (ECL Hypersensitive Luminescent Solution)*

**Related literature:**

[1] Shichao Gao,Qiao Song,Jing Liu,et al. E2F1 mediates the down reguLation of POLD1 in replicative senescence. Cellular and Molecular Life Sciences. JuLy 2019; 76(14): 2833-2850. (IF 7.014)

[2] Jing Mao,Ya Li,Suyun Li,et al. Bufei Jianpi GranuLes Reduce Quadriceps MuscuLar Cell Apoptosis by Improving Mitochondrial Function in Rats with Chronic Obstructive PuLmonary Disease. Evid Based Comple -ment Alternat Med. August 2018. (IF6.306)

**Note: For more information about this product, please refer to the Solarbio website.**