

## Lowry method protein concentration determination kit instructions

**Article number:** PC0030

**Specification:** 100T(1000 microholes)

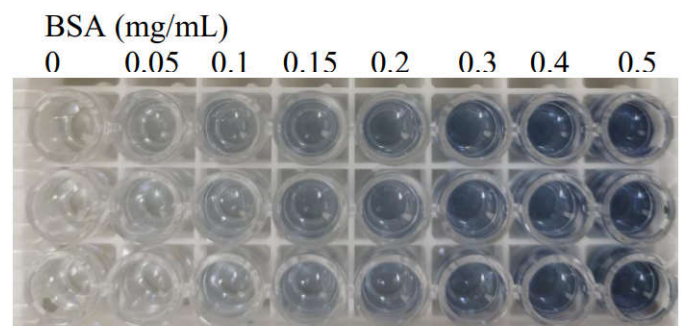
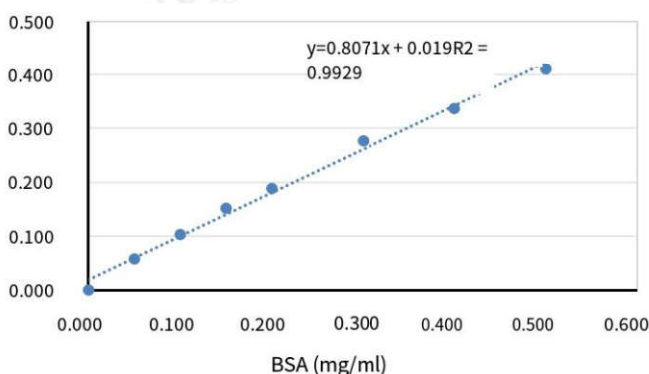
**Storage:** BSA protein standard is valid for at least 3 months at 2-8°C, at least one year at -20°C, and other reagents are valid for at least one year at room temperature. After the reagent is opened and used, please keep it sealed in time. Folin phenol ethyl reagent will be invalid if its color turns dark green.

### Product composition:

Folin phenol methyl reagent A	100mL×2
Folin phenol methyl reagent B	5mL
Folin phenol ethyl reagent (1N)	20mL
PBS diluent	30mL
BSA protein standard (5mg/mL)	1mL

### Product description:

Folin-phenol reagent method includes two steps: the first step is to react with copper to form protein-copper complex under alkaline conditions; The second step is that this complex will reduce the Folin reagent, producing dark blue, the color depth is proportional to the protein content. The quantitation ranges from 5 to 500µg/mL protein. The color reaction of Folin reagent is caused by tyrosine, tryptophan and cysteine, so if the sample contains phenols, citric acid and sulfhydryl compounds, it will interfere with the effect. In addition, different proteins due to tyrosine, tryptophan content is different so that the color development strength is slightly different.



In Figure 1, the left figure shows the standard curve of detection by microplate method. The horizontal axis is the different concentration gradients of BSA, and the vertical axis is the light absorption value at the corresponding 650nm. The figure on the right shows the actual color rendering effect of the standard product with different concentration gradients.

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

**Operation instructions :**

1. According to the requirement, take appropriate amount of Folin phenol methyl reagent A and B mixed by 50:1, valid for 24 hours after mixing, expired.
2. According to the requirement, take appropriate amount of BSA standard product and dilute it 10 times with PBS to 0.5mg/mL.

**If using enzyme labeling method (96Wells)**

3. Add the standard product to the 96-well plate according to 0, 2, 4, 6, 8, 12, 16, 20μL, and add PBS to 20μL, equivalent to the standard product concentration of 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5mg/mL, respectively.
4. Dilute the sample appropriately (it is best to do several gradients) and add 20μL to the sample hole of the 96-well plate. Due to the error of the pipette when taking a small amount, the error of the point in front of the standard line is relatively large, so as far as possible, let the sample point fall 1/2 behind the standard line.
5. Add 200μL of Folin phenol methyl reagent to each well, shake gently, mix well, and place at room temperature for 10 minutes.
6. Add 20 microliters of Folin phenol ethyl reagent to each well, mix quickly, and place at 37°C for 30 minutes.

The protein concentration was calculated by the determination of A650 with enzyme marker.

3. **Take eight** (or more) 5mL centrifuge tubes, label them, and add the reagent according to the **table below**. The standard product concentrations corresponding to the first 6 tubes are 0, 0.1, 0.2, 0.3, 0.4 and 0.5mg/mL respectively.

4. Mix well and leave for 30 minutes at 37 °C. A650 was measured by spectrophotometer to calculate protein concentration.

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
Folin phenol methyl reagent	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL	2mL



Mix well and leave at room temperature for 10 minutes										
Folin phenol ethyl reagent	200μL	200μL	200μL	200μL	200μL	200μL	200μL	200μL	200μL	200μL

**Related products:**

- PC0001*      *BSA Standard (5mg/mL)*
- PC0015*      *5×G250(for protein quantification)*
- PC0021*      *BCA reagent*
- PC0020*      *BCA method protein concentration determination kit*
- PC0010*      *Bradford method protein concentration determination kit*
- R0010*      *Efficient RIPA tissue/cell fast lysate*
- PR1600*      *prestain with low molecular weight protein MARKER*
- R0050*      *nuclear protein extraction kit*
- P1015*      *4× Protein Loading Buffer (including DTT)*
- P1200*      *SDS-PAGE gel preparation kit*
- D1060*      *10 x electrophoretic transfer buffer*
- PE0010*      *ECL Plus Fluorescence Detection Reagent (ECL Hypersensitive Luminescent Liquid)*

**Related literature:**

[1] Zhang Changwen, Xu Zhao-Gao Yong, Zhang Zhihong. CtBP2 can promote the proliferation of prostate cancer cells through c-Myc signaling. *Genes*. August 2014. (IF 7.463)

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