

## Folin-phenol reagent preparation

**Cat:** F8060

**Size:** 50mL/100mL

**Storage:** Store at room temperature away from light, valid for 3 years; It is recommended to store at 4°C after opening.

### Product parameters:

English name: Folin & Ciocalteu's phenol reagent

Alias: Folin-Phenol

Appearance (character): Light yellow liquid

### Protocols:

#### 1, Folin-phenol reagent A (customers need to prepare or choose the corresponding kit) :

Dissolve 1g  $\text{Na}_2\text{CO}_3$  in 50mL 0.2mol/L NaOH, and then dissolve 0.5g (copper sulfate)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100mL 1% potassium sodium tartrate (or sodium tartrate) solution, and then mix the former 50mL with the latter 1mL, now used, this reagent can only be used for one day, expired.

#### 2. Folin-phenol reagent B:

The concentration of this reagent is 1mol/L, which is the application liquid of Folin-phenol reagent. Folin-phenol reagent should be sealed and stored in a refrigerator at 4°C away from light.

#### 3, Folin-phenol method determination principle:

Under alkaline conditions, protein reacts with copper to form protein-copper complex. This complex reduces the reagent phosphomolybdate - phosphotungstic acid (Folin reagent), the mixture of dark blue (phosphomolybdate blue and phosphotungstic blue mixture), the color is proportional to the protein content. This method is simple and easy to operate, the sensitivity is 100 times higher than biuret method, and the quantitative range is 5 ~ 100 $\mu\text{g}$  protein.

#### Operation method (for reference only) :

##### 1. the production of standard curve:

Take 14 test tubes and divide them into two groups. Add 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1mL standard protein solution (250 $\mu\text{g}/\text{mL}$ ), fill up to 1mL with water, add 5mL reagent A, mix well, place at 20-25°C for 10 minutes, then add 0.5mL reagent B, shake well immediately. Heat at 20-25°C for 30 minutes, and then colorimetric at 500nm. Determine the optical density value, take the average value of the two groups of determination, take the protein concentration as the horizontal coordinate, the optical density value as the vertical coordinate, draw the standard curve value as the quantitative basis.

##### 2. Sample determination:

Take 1mL sample solution (about 20-250 $\mu\text{g}$ / peptide or protein) and add 5mL reagent A to mix, place at 20-25°C for 10 minutes, then add 0.5mL reagent B (Folin-phenol), immediately shake well, hold at 20-25°C for 30 minutes, and then compare colors at 500nm, with 1mL water instead of the sample as a blank control. After determination, the concentration of the unknown sample can be found in the standard curve. If the colorimetric cup with 0.5cm optical path is used for colorimetric comparison, the following method can be performed: take

0.2mL sample solution (containing about 5-100 $\mu\text{g}$  polypeptide or protein) and add 1mL reagent A (a small test

tube with a diameter of 0.3-0.5cm can be selected) and mix, 10 minutes later, then add 0.1mL reagent B, immediately mix, 30 minutes later, colorimetric. In general, if the concentration of polypeptide or protein is 2-25 $\mu$ g, the wavelength of measurement is 755nm, and more than 25 $\mu$ g, 500nm colorimetric is appropriate.

**Note:**

1. Folin reagent color reaction is caused by tyrosine, tryptophan, cysteine, so if the sample contains phenols, citric acid and sulfhydryl compounds, there is interference.
2. Folin phenol is only stable under acidic conditions, so it should be mixed immediately after adding reagent B, and the reaction should be completed before reagent B is destroyed, otherwise the degree of color development will be weakened.
3. The determination of protein concentration by this method will be affected by the specific effect of protein, that is, different proteins due to the different content of tyrosine and tryptophan make the color intensity slightly different, and the standard curve is not strictly in a straight line form.

**Related products:**

*F8010 folinol*

*PC0020 BCA protein concentration determination kit*

*PC0010 Bradford protein concentration determination kit*

*PC0030 Lowry Assay kit for protein concentration -1000 microhole*