

# Betaine Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC3130

**Size:** 50T/48S

## Components:

Extract solution: 80% methanol, self-provided reagent. Take 40 mL of methanol and add 10 mL of distilled water;

Reagent I: Powder ×5, stored at 4°C. Prepared according to the dosage before use, add 15 mL of distilled water to each bottle, adjust the pH to 1 with concentrated HCl, stir for 45 minutes. After filtration, After filtering, making up to 20mL with distilled water.

Reagent II: Ether, self-prepared reagent.

Reagent III: 100 mL×1, stored at 4°C.

Reagent IV: Powder×1, stored at room temperature.

Standard: Powder×1. Add 3 mL of distilled water before use to obtain 10 mg/mL betaine standard solution.

## Product Description

Betaine is a kind of quaternary ammonium type water-soluble alkaloid widely distributed in animals, plants and microorganisms. It is the oxidation product of choline in organism. It can enhance immunity, reduce blood lipid, resist oxidation and anti-tumor. It can also be used as a methyl donor to promote protein and fat metabolism, increase appetite, relieve stress, regulate osmotic pressure, stabilize vitamins and other biological functions It is widely used in chemical industry, medicine, food additive and other fields.

Under strong acid conditions, betaine reacts with Raynaud salt to produce precipitation. The precipitation is dissolved in acetone to form a red solution. There is a characteristic absorption peak at 525 nm. The absorption value at 525 nm is determined to obtain the content of betaine in the sample.

## Reagents and Equipment Required but Not Provided.

Table type centrifuge, spectrophotometer, water-bath, 1 mL glass cuvette, transferpettor, mortar/homogenizer, methanol, ether, Concentrated hydrochloric acid (37% HCl) and distilled water.

## Procedure

1. Extraction of sample

a. Bacteria or cell treatment:

Take about 0.2 g of sample passing 40 mesh sieve after drying, add 1 mL of Extract solution, and extract it at 60°C for 30 minutes, shake continuously in the meantime. Add about 3 mg of Reagent IV with forceps and shake it fully. Centrifugated at 10000 rpm for 15 minutes at 25°C, take the supernatant, volatilize methanol at 70°C (about 0.2 mL is left, methanol must be volatilized

completely), and then the constant volume of water is 1 mL.

## 2. Measurement steps

- a. Preheat the spectrophotometer for 30 minutes, adjust the wavelength to 525 nm and adjust zero with Reagent III.
- b. Dilute 10 mg/mL betaine standard solution to 9, 8, 7, 6, 5, 4, 3 and 2 mg/mL standard solution for standby.
- c. Operation table:

Reagent name (mL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	0.1	-	-
Standard solution	-	0.1	-
Distilled water	-	-	0.1
Reagent I	1	1	1
Mix well, react at 4°C for 2 hours, centrifugated at 8000 rpm for 15 minutes at 25°C, and discard the supernatant.			
Reagent II	1	1	1
Centrifugated at 8000 rpm for 10 minutes at 25°C, discard the supernatant. Put it in the fume hood to make Reagent II volatilize naturally to complete.			
Reagent III	1	1	1
Shake to make the precipitate fully dissolved, measure $A_S$ , $A_T$ and $A_B$ , calculate $\Delta A_S = A_S - A_B$ , $\Delta A_T = A_T - A_B$ .			

## Calculation of Betaine Content:

### 1. Drawing of standard curve:

Take  $\Delta A_S$  as y-axis, standard solution concentration as x-axis, draw standard curve, get standard equation  $y = kx + b$ , bring  $\Delta A_T$  into the equation, get x (mg/mL).

### 2. Calculation of cellulose content:

Betaine content (mg/g) =  $x \times V_{EV} \div W$ .

$V_{EV}$ : Volume of extract solution, 1 mL;

W: Mass of sample drying, g.

### Note:

1. When the Reagent I is prepared, the pH shall be strictly controlled to 1, otherwise the reaction will be incomplete. After preparation, it can only be stable at 4°C for 48 hours, so it can be used as soon as possible after preparation.
2. Reagent II and III have certain irritation to respiratory tract, please do a good job of protection.
3. When  $\Delta A$  is greater than 0.4, it is recommended to dilute the sample for determination.
4. The detection range of this kit is between 2 mg/mL and 9 mg/mL.

## Recent Product Citations:

[1] Yanan Wang, Chengzhen Liang, Zhigang Meng, et al. Leveraging Atriplex hortensis choline

monooxygenase to improve chilling tolerance in cotton. Environmental and Experimental Botany.  
June 2019;162:364-373.(IF3.712)

**Related Products:**

BC2030/BC2035 Isocitrate Lyase(ICL) Activity Assay Kit  
BC3170/BC3175 Acetokinase(ACK) Activity Assay Kit  
BC2010/BC2015 Glycollic Oxidase Activity Assay Kit