Betaine Content Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/microplate reader
Catalog Number: BC3135
Size:100T/96S

Components:
Extract solution: 80% methanol, self-provided reagent. Take 80 mL of methanol and add 20 mL of distilled water.
Reagent I: Powder×4, stored at 4℃. 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, making up to 20 mL with distilled water.
Reagent II: Ether, self-prepared reagent.
Reagent III: 40 mL×1, stored at 4℃.
Reagent IV: Powder×1, stored at room temperature.
Standard: Powder×1, stored at 4℃. Add 1 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/microplate reader, water-bath, micro glass cuvette/96 well flat-bottom plate, transferpettor, mortar/homogenizer, methanol, ether, HCl, 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℃ 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℃, take the supernatant, volatilize methanol at 70℃ (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/microplate reader 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	0.03	-	-
Standard solution	-	0.03	-
Distilled water	-	-	0.03
Reagent I	0.3	0.3	0.3
Mix well, react at 4℃ for 2 hours, centrifugated at 8000 rpm for 15 minutes at 25℃, and discard the supernatant.			
Reagent II	0.3	0.3	0.3
Centrifugated at 8000 rpm for 10 minutes at 25℃, discard the supernatant. Put it in the fume hood to make Reagent II volatilize naturally to complete.			
Reagent III	0.3	0.3	0.3
Shake to make the precipitate fully dissolved, take 0.2 mL in micro glass cuvette/96 well plate to 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 ΔA_S as y-axis, standard solution concentration as x-axis, draw standard curve, get standard equation y = kx+b, bring ΔA_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℃ 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. Make the range of ΔA within the range of standard curve.
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