Oxalic Acid Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4365

Size: 100T/96S

Components:

Reagent I: Powder×1, store at 4℃. Add 3 mL distilled water before use. Mix thoroughly;

Reagent II: Liquid 20 mL×1, store at 4℃;

Reagent III: Liquid 1.5 mL×1, store at 4℃ and protect from light;

Reagent IV: Powder×1, store at 4℃;

Standard: Powder×1, store at 4℃. Add 792 μL distilled water to prepare 100 μmol/mL oxalic acid standard solution before use.

Product Description:

Oxalic acid is a kind of dicarboxylic acid. It widely exists in the plant kingdom. It has different functions in different fields. In medicine, printing and dyeing, plastics and other industrial production, oxalic acid can be used as pharmaceutical raw materials, complexing agents, bleaching agents, precipitants and reducing agents. From the perspective of food, long-term consumption of vegetables with high oxalic acid content is easy to cause arthritis, low blood calcium, bladder stones, kidney stones and other diseases. Therefore, it is considered to be an antagonist of mineral elements.

Fe^{3+} can form purple complex with sulfosalicylic acid at pH is 2. It has a characteristic absorption peak at 510 nm. Fe^{3+} and sulfosalicylic acid can produce a purple complex. Oxalic acid and oxalate can make it lighter. At the same time, the absorbance of Fe^{3+} and sulfosalicylic acid complex decreases with the increase of oxalic acid. Accordingly, the content of oxalic acid in the sample can be calculated from the reduced absorbance value.

Required but Not Provided:

Spectrophotometer/ microplate reader, desk centrifuge, water-bath/constant temperature incubator, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, EP tube, ice and distilled water.

Protocol

I. Preparation:

Tissue: according to the tissue weight (g): the volume of the distilled water (mL) is 1:5-10. It is suggested that add 1 mL of distilled water to 0.1 g of tissue. Homogenize on ice. Then add a little reagent IV (about 3-5 mg). After shaking and mixing, put it into a 75℃-water bath to decolorize for 30 min. Shake 2-3 times during this period. Centrifuge at 3000 rpm for 15 minutes at room temperature after decolorization. Take the supernatant on ice for test. (If the tissue precipitation and reagent IV cannot be removed by one-time centrifugation. It is suggested that the supernatant be

centrifuged several times; If the primary decolorization is incomplete, it is recommended to decolorize repeatedly until the solution is colorless or slightly milky white).

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 510 nm, set the counter to zero with distilled water.
2. Dilute 100μmol/mL oxalic acid standard solution with distilled water to 18、15、12、6、3、1.5、0.75、0.375μmol/mL standard solution for standby.
3. Operation table:

Reagent Name (μL)	Blank tube (A_B)	Test tube (A_T)	Standard tube (A_S)
Reagent I	20	20	20
Reagent II	150	150	150
Reagent III	10	10	10
Distilled water	20	-	-
Sample	-	20	-
Standard	-	-	20
Mix thoroughly. Standing at room temperature for 20 min.			
Add the reaction solution to the micro glass cuvette/96 well flat-bottom plate, and measure the absorption value A at 510 nm. Record as A_B, A_T, A_S. $\Delta A_S=A_B-A_S$. $\Delta A=A_B-A_T$. Blank tube and standard tube only need to test once or twice in the same batch.			

Note: If the sample size is large. According to the proportion of reagent I: reagent II: reagent III = 2:15:1 (V: V: V) required by the sample to prepare the working solution. The sample adding system is 180μL working solution and 20μL sample (distilled water or standard solution).

III. Oxalate Calculation:

1. Drawing of standard curve

Take the concentration of each standard solution as the x-axis, and the corresponding ΔA standard as the y-axis, and the linear regression equation y=kx+b is obtained. Bring ΔA into the equation to get x (μ mol/mL).

2. Calculate:

Oxalic Acid (mg/g weight) $=x\times V_E\times M\times 10^{-3}\div W=0.09x\div W$

V_E: Extract solution volume, 1 mL;

W: Sample weight, g;

M: Molecular weight of oxalic acid，90.04；

10^{-3}: Unit conversion factor, 1 μg=10^{-3} mg。

Note:

1. When the determination of Δ A is greater than 1, it is recommended to dilute the sample with distilled water before the determination, and multiply the dilution times in the calculation formula.
2. If reagent I and II are combined with reagent III to form working solution for use, it needs to be

prepared according to the number of the sample. Do not prepare it all at once.

Related products：

BC4180/BC4185	Shikimic acid Dehydrogenase(SD) Activity Assay Kit
BC4200/BC4205	Lignin Activity Assay Kit
BC4220/BC4225	4-Coumarate CoA Ligase(4CL) Activity Assay Kit
BC1140/BC1145	Creatine Kinase(CK) Activity Assay Kit

Technical Specifications：

Minimum Detection Limit：0.1601 μmol/mL

Linear Range：0.1875-18 μmol/mL