CheKine™ Micro Amylose Content Assay Kit

Cat #: KTB3017 Size: 48 T/48 S 96 T/96 S

	Micro Amylose Content Assay Kit		
REF	**Cat #:** KTB3017	LOT	**Lot #:** Refer to product label
	Detection range: 0.05-0.8 mg/mL		**Sensitivity:** 0.05 mg/mL
	Applicable sample: Plant Tissue		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Amylose is a polysaccharide chain composed of D-glucose units linked by α-(1,4) glycosidic bonds. Its quantification is of great significance for evaluating the nutritional value of food and investigating sugar metabolism in plants. CheKine™ Micro Amylose Content Assay Kit provides a simple, convenient, and rapid method for detecting amylose content, suitable for plant samples. The principle is based on the use of 80% ethanol to separate soluble sugars from starch in the sample. The complex formed by amylose and iodine exhibits an absorption peak at 620 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	90 mL	90 mL×2	4℃
Reagent Ⅱ	1 mL	2 mL	4℃
Reagent Ⅲ	0.2 mL	0.4 mL	4℃, protected from light
Reagent Ⅳ	1 mL	1 mL	4℃
Standard	Powder×1 vial	Powder×1 vial	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 620 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, vortex oscillator, vortex mixer, centrifuge
- Deionized water, absolute ethanol, 80% ethanol, ethyl ether
- Homogenizer

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard: Prepared before use. Add 0.1 mL of absolute ethanol and 0.9 mL of Reagent IV, mix well, and seal the container with a sealing film. Heat in a boiling water bath until dissolved to prepare a 10 mg/mL amylose standard solution. Unused reagents can be stored at 4℃ for up to one month.

1 mg/mL amylose standard solution: Take 100 μL of the 10 mg/mL amylose standard solution and add 900 μL of Reagent I to prepare a 1 mg/mL amylose standard solution. Using the 1 mg/mL amylose standard solution, further dilute the standards according to the table belo:

Num.	Standard Volume (μL)	Reagent I (μL)	Concentration (mg/mL)
Std.1	240 μL of 1 mg/mL Standard	60	0.8
Std.2	180 μL of 1 mg/mL Standard	120	0.6
Std.3	120 μL of 1 mg/mL Standard	180	0.4
Std.4	60 μL of 1 mg/mL Standard	240	0.2
Std.5	30 μL of 1 mg/mL Standard	270	0.1
Std.6	15 μL of 1 mg/mL Standard	285	0.05
Blank	0	300	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended. If the experiment is not conducted immediately, samples can be stored at -80℃ for up to one month. During measurement, the thawing temperature and time should be controlled. If thawing at room temperature, the sample must be completely thawed within 4 h.

After drying the sample in an 80℃ oven, weigh 0.01 g of the dried sample and grind it in a mortar. Add 1 mL of 80% ethanol, homogenize thoroughly, and transfer to an EP tube. Extract in an 80℃ water bath for 30 min, then centrifuge at 3,000 g for 5 min at 25℃. Discard the supernatant and retain the pellet. Add 1 mL of diethyl ether, vortex for 5 min, and centrifuge again at 3,000 g for 5 min at 25℃. Discard the supernatant and retain the pellet. Add 1 mL of Reagent I to dissolve the pellet completely, heat in a 90 ℃ water bath for 10 min, cool, and centrifuge at 3,000 g for 5 min at 25 ℃. Collect the supernatant for subsequent measurement.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 620 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in a 96-well microplate or microglass cuvette):

Reagent	Test Well (μL)	Blank Well (μL)
Supernatant	20	0
Standard	0	20

Reagent Ⅱ	14	14
Deionized water	120	120
Reagent Ⅲ	2	2
Deionized water	44	44

Mix well, measure the absorbance at 620 nm, and record the values as A_{Test}, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank and Standard wells need to be tested only 1-2 times. If there are many samples to be tested, a working solution can be prepared according to the ratio of Reagent II: Reagent III: deionized water = 14: 2: 144 based on experimental requirements, and the solution should be prepared fresh before use. Before the experiment, it is recommended to select 2-3 samples with expected significant differences for a preliminary test. If ΔA_{Test} is less than the $\Delta A_{Standard}$ of 0.05 mg/mL, the sample amount can be appropriately increased. If ΔA_{Test} exceeds the $\Delta A_{Standard}$ of 0.8 mg/mL, the supernatant can be further diluted with Reagent Ⅰ. Multiply the final result by the dilution factor, or reduce the amount of sample used for extraction.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (mg/mL).

2. Calculation of amylose content

Calculated based on the dry weight of the sample:

Amylose content (mg/g dry weight)=$(x\times V_1)\div(W\times V_1\div V_2)$**=x÷W×F**

V_1: Volume of the sample added to the reaction system, 0.02 mL; V_2: Volume of Reagent Ⅰ added, 1 mL; W: Sample mass, g; F: Dilution factor.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

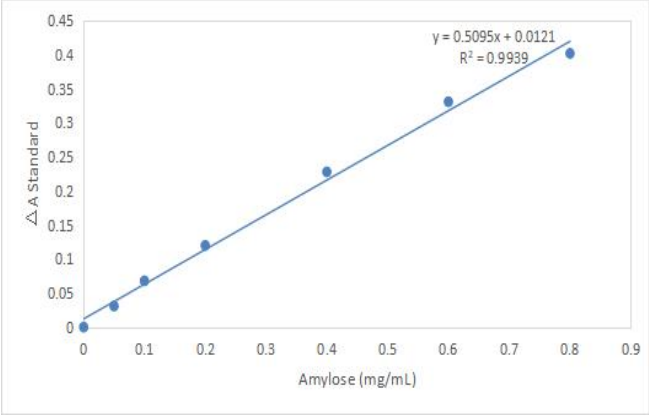

Figure 1. Standard curve of amylose activity.

Examples:

Take 0.01 g of wheat that has been oven-dried at 80℃, ground into powder, and analyze according to the above steps, after an 5-fold dilution, use 96-well plate to calculate ΔA_{Test}=0.214-0.055=0.159, x=332. The content calculated according to the dry weight of the sample is as follows:

Amylose content (mg/g dry weight)=0.332÷0.01×5=166 mg/gmg/g dry weight.

Recommended Products

Catalog No.	Product Name
KTB1371	CheKine™ Micro Starch Assay Kit
KTB1370	CheKine™ Micro α-Amylase Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.