UDP-glycose flavonoid glycosyltransferase (UFGT)Activity Assay Kit

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

Operation Equipment: Ultraviolet spectrophotometer/ Microplate Reader

Cat No: BC5665

Size: 100T/96S

Components:

Extract: Liquid 110 mL ×1. Storage at 2-8℃. Pour the powder I into the extract before use. This solution is a suspension, shake well before use

Powder I： Powder×1. Store at 2-8℃.

Reagent I : Liquid 40 mL ×1. Store at 2-8℃.

Reagent II: Liquid 1mL ×1. Store at -20℃.

Reagent II working solution: before use according to the number of samples in accordance with the reagent I: reagent II = 171μL: 9μL（180μL，2T）ratio of the preparation, mix thoroughly, ready to use;

Reagent III: Powder×1. Store at -20℃. Add 10 mL of distilled water to fully dissolve before use. Store unused reagents in separate packages at -20℃ for up to 4 weeks, avoid repeated freezing and thawing;

Reagent IV: Liquid 80 μ L ×1. Store at 2-8℃.

Reagent V: Liquid 35 μ L ×1. Store at 2-8℃.

Reagent VI: Powder×1. Store at -20℃. Add 6 mL of distilled water to fully dissolve before use. Store unused reagents in separate packages at -20℃ for up to 4 weeks, avoid repeated freezing and thawing;

Reagent VII: Powder×1. Store at -20℃. Reagent is placed in reagent bottles in glass vials. Add 6 mL of distilled water to fully dissolve before use. Store unused reagents in separate packages at -20℃ for up to 4 weeks, avoid repeated freezing and thawing;

Working solution: before use according to the number of samples in accordance with the reagent I: reagent IV: reagent V: reagent VI: reagent VII = 1.4 mL: 5 μL: 2 μL: 0.3 mL: 0.3 mL (2007 μL, about 7T) of the proportion of the preparation, fully mixed, ready to use. (Reagents IV and V should be used by centrifuging the liquid to the bottom before use)

Product Description:

UDP-glycose flavonoid glycosyltransferase (UFGT) is the last acting enzyme of the shikimate pathway and the first acting enzyme that causes the formation of stable anthocyanins and their conversion from colorless to colored; UFGT is the key enzyme in the process of fruit coloring, which converts unstable anthocyanins to stable anthocyanin.

UFGT catalyzes the formation of UDP and quercetin glycosides from UDPG and quercetin; UDP oxidizes NADH to NAD^+ under the action of pyruvate kinase and lactate dehydrogenase, and the rate of

NAD^+ generation is directly proportional to the content of UDP, which is reflected by the rate of decrease of absorbance at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/ microplate reader, cryogenic centrifuge, water bath/incubator, analytical balance, adjustable pipette, micro quartz cuvette/96-well UV plate, mortar/homogenizer, distilled water and ice.

Procedure:

Sample preparation:

Tissue: according to the ratio of tissue mass (g): volume of extract (mL) is 1:5~10 (it is recommended to weigh about 0.1g of tissue and add 1mL of extract), homogenize in an ice bath. 10,000g centrifugation at 4 ℃ for 10min, take the supernatant, and put it on ice to be measured..

Determination procedure:

1. Preheat ultraviolet spectrophotometer / microplate reader for 30 minutes, adjust the wavelength to 340 nm, ultraviolet spectrophotometer set zero with distilled water.
2. Add samples in 1.5 mLEP tubes as follows .

Reagent name (μL)	Test tube(T)	Control tube(C)
Sample	20	-
Distilled water	-	20
Reagent II working solution:	90	90
Reagent III	90	90
Mix well, react at 30℃ for 4h, inactivate at 95℃ for 10 min, cool to room temperature, centrifuge at 10,000g for 5min at 4℃, and take the supernatant to be tested. (During this period, preheat the working solution at 37℃ for 5min)		
Supernatant	30	30
Working solution	270	270
Add the supernatant and working solution into 1mL quartz cuvette respectively, mix well immediately and then measure the absorbance value A1 at 340nm at 10s, put it into 37℃ water bath or constant temperature incubator for 2min, take it out and wipe it dry quickly to measure the absorbance value A2 at 2min10s, and then record the absorbance value A1 at 10s and absorbance value A2 at 340nm after 2min. Calculate At= A1t- A2t, Ab = A1b- A2 b, ΔA = At - Ab. Blank tubes should only be done 1-2 times.		

Calculation:

micro quartz cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol NADH per hour every milligram of protein.

UFGT activity (U/mg prot) = ΔA×V**rII**÷（ε×d）×10^9÷ （Cpr ×Vs÷V**rI**×Vsu）÷T×F

=4019.29×ΔA ÷Cpr×F

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 nmol NADH per hour every gram of tissue.

UFGT activity（U/g weight）= ΔA×V**rII**÷（ε×d）×10^9÷（W× Ve÷Vs÷V**rI**×Vsu）÷T×F

=4019.29×ΔA÷W×F

V**rI**： Total volume of reaction system in the first step at 30°C (V sample + V reagent II working solution + V reagent III)， 0.2 mL； V**rII**： 37°C Total volume of the second step reaction system，$0.3×10^{-3}$ L； ε：NADPHmolar absorptivity，$6.22×10^3$ L/mol/cm；d：micro quartz cuvette optical path，1cm；Vs：Volume of sample added，0.02mL；Vsu：Volume of supernatant in the second step of the reaction，0.03 mL；Ve：Volume of extract added，1 mL；T：reaction time，4h；Cpr：concentration of protein，mg/mL；W：weight of the sample，g；F：sample dilution times；10^9：conversion factor，1mol=10^9nmol。

96-well UV plate

Replace d=1cm in the above formula with d=0.6cm (96-well UV plate optical path) for calculation.

Note:

1. If A1t< A1b or ΔA is greater than 0.5, dilute the supernatant or shorten the reaction time at 30°C and remeasure; if ΔA is less than 0.005, increase the sample volume or extend the reaction time at 30°C and remeasure. The formula was modified synchronously for the final calculation.

Experimental example:

1. 1. Weigh 0.1078g of eustoma, add the extract for ice bath homogenization, operate according to the assay steps, measured with a micro quartz cuvette. Calculation At= A1t - A2t= 0.9988-0.9807=0.0181, Ab= A1b - A2b =0.8157-0.8121=0.0036, ΔA = At- Ab =0.0145，bring in the formulae to calculate:

   UFGT activity（U/g weight） = 4019.29 × ΔA÷W = 540.63 U/g mass

2. Weigh 0.1133g of onion, add the extract solution to the ice bath homogenization, follow the steps of the assay, measured with a micro quartz cuvette to calculate the At= A1t - A2t =0.8309-0.8157=0.0152, Ab= A1b - A2b =0.8157-0.8121=0.0036, ΔA = At- Ab = 0.0116,bring in the formulae to calculate:

   UFGT activity（U/g weight） = 4019.29 × ΔA÷W = 411.51 U/g mass

References:

[1] Parvaneh, TaherehAbedi, BahramDavarynejad, Gholam HosseinMoghadam, Ebrahim Ganji. Enzyme activity, phenolic and flavonoid compounds in leaves of Iranian red flesh apple cultivars grown on different rootstocks[J]. Scientia horticulturae, 2019, 246.

[2] Mori K, Sugaya S, Gemma H. Decreased anthocyanin biosynthesis in grape berries grown

under elevated night temperature condition[J]. Hort, 2005, 105(3):319-330.

Related Products:

BC1350/BC1355 Plant Proanthocyanidins Assay Kit
BC4090/BC4095 Anthocyanin Reductase Activity Assay Kit

BC1380/BC1385 Anthocyanin Content of Plant Assay Kit

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