Dihydroflavonol reductase (DFR) activity Assay Kit

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

Operation Equipment: Microplate Reader/ Ultraviolet spectrophotometer

Cat No: BC5785

Size: 100T/96S

Components:

Extract: Liquid 30mL×1. Store at 2-8℃. Before use, the extract is diluted 10 times with distilled water and then used, and the diluted extract can be stored at 2-8℃ for 8 weeks.

Reagent I: Powder×1. Store at 2-8℃.

Reagent II: Powder×1. Store at 2-8℃.

Reagent III: Powder×1. Store at 2-8℃.

Reagent IV: Liquid 15mL×1. Store at 2-8℃.

Reagent V: Powder×1. Store at -20℃. Reagents are placed in the bottle in a glass tube. Before use, add 6.25mL distilled water to fully dissolve, the dissolved reagent can be stored at -20℃ for 4 weeks, avoid repeated freezing and thawing.

Mixing solution: Before use, dissolve reagent I, reagent II and reagent III together in 1mL of anhydrous ethanol to fully dissolve the reagent after dissolution, the reagent can be stored at 2-8℃ for 4 weeks.

Working solution: Before use according to the sample volume according to the mixture: distilled water =5μL:495μL（500μL，25T）ratio, ready to use.

Product Description:

Dihydroflavonol-4-reductase (DFR) is a key enzyme in the biosynthetic pathway of phycocyanin, which catalyzes the production of different anthocyanin precursors from dihydroflavonols. Therefore, DFR determines the type and content of anthocyanins in plants, which in turn affects the color of the tissues or organs of plants.

DFR, NADPH and dihydroflavonols react to produce anthocyanin precursors. NADPH has a maximum absorption peak at 340 nm. The magnitude of DFR activity can be obtained by detecting the rate of change of NADPH at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet Spectrophotometer/ microplate reader, water bath, benchtop centrifuge, balance, adjustable pipettes, micro quartz cuvette/96 well UV plate, mortar and homogenizer, anhydrous ethanol (AR), ammonium sulfate (AR), ice and distilled water.

Procedure:

Sample preparation:

(1) Homogenize in an ice bath at a ratio of 1:5~10 tissue mass (g): extract volume (mL) (it is recommended to weigh about 0.2g of tissue and add 2mL of extract), centrifuge at 12000g for 15min

at 4℃, take 1mL of supernatant and transfer it to a new 2mL EP tube.

(2) Add about 0.5g of ammonium sulfate to the supernatant, fully dissolve it and leave it at 4°C for 2 h. Then centrifuge at 12000g for 15min at 4°C and discard the supernatant.

(3) Add 0.4mL of extraction solution to the precipitate, dissolve it thoroughly and put this precipitate solution as a sample on ice for testing.

Determination procedure:

1. Preheat ultraviolet Spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 340 nm, spectrophotometer set zero with distilled water.
2. Preheat the reagent IV at 30℃ for 15min before use.
3. Add samples in micro quartz cuvette/96 well UV plate as follows .

Reagent name (μL)	Test tube(T)	Control tube(C)
Sample	40	-
Distilled water	-	40
working solution	20	20
Reagent IV	140	140
Reagent V	20	20
Immediately mix well and determine the absorbance value A1 at 340nm at 10s, quickly placed in a 30℃ water bath or constant temperature incubator reaction for 30min, take out and quickly wipe dry to determine the absorbance value A2 at 30min10s, record the absorbance value A1 at 10s at 340nm and the absorbance value A2 after 30min, Calculate the ΔAt= A1t- A2t, ΔAb = A1b - A2b, ΔA = ΔAt- ΔAb. (Blank tubes only need to be done 1-2 times)		

Calculation:

A micro quartz cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol NADPH per minute every milligram of protein.

DFR activity (U/mg prot) ΔA÷（ε×d）×Vt×10^9÷（Cpr×V 样）÷T=29.475×ΔA÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the the amount of enzyme catalyzes the consumption of 1 nmol NADPH per minute every gram of tissue.

DFR activity (U/g mass) =ΔA÷（ε×d）×Vt×10^9÷（W÷2÷Ve×Vs）÷T=23.58×ΔA÷W

ε：NADPH molar extinction coefficient，6.22×10^3L/mol/cm；d：quartz cuvette optical path，1cm；Vt：Total volume of reaction system，1100μL=1.1×10^{-3} L；10^9：conversion factor，1mol=10^9nmol；Vs：Volume of sample added，200μL=0.2mL；Ve：Total sample volume，0.4mL；2：the precipitation of pretreatment (3) is half of the homogenization of pretreatment (1)；T：reaction time，30min；W：weight of the sample，g； Cpr：concentration of protein，mg/Ml

B 96 well UV plate

Just change d-1cm to d-0.6cm (96-well UV plate optical diameter) in the above formula for calculation.

Note:

1. If ΔA > 1 or A1t < A1b, you can dilute the sample (precipitation dissolution in pretreatment) with extraction solution (dilution) or shorten the reaction time at 30°C. If ΔA is less than 0.01, you can increase the sample volume or extend the reaction time at 30°C. Synchronize the modification of the calculation formula for the final calculation.

Experimental example:

1. Weigh 0.2152g sunflower, add the extract for ice bath homogenization, follow the assay steps, measured with 96-well UV plate to calculate ΔAt=0.916-0.833 =0.083， ΔAb=0.723-0.700=0.023 。 ΔA =ΔAt-ΔAb=0.06，brought to the formula:

   DFR activity (U/g mass) = 23.58 ÷0.6（96-well UV plate optical diameter）× ΔA ÷ W =10.957 U/g mass

References:

[1] Hayashi M, Takahashi H, Tamura K, et al. Enhanced dihydroflavonol-4-reductase activity and NAD homeostasis leading to cell death tolerance in transgenic rice[J]. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102(19):p.7020-7025.

[2] Petit P, Granier T, Béatrice Langlois d'Estaintot, et al. Crystal Structure of Grape Dihydroflavonol 4-Reductase, a Key Enzyme in Flavonoid Biosynthesis[J]. Journal of Molecular Biology, 2007, 368(5):1345-1357.

Related Products:

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