

Dihydroflavonol reductase (DFR) activity Assay Kit

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

Operation Equipment: Microplate Reader/ Ultraviolet spectrophotometer

Cat No: BC5785

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract Solution	Liquid 30 mL×1	2-8°C
Reagent I	Powder×1	2-8°C
Reagent II	Powder×1	2-8°C
Reagent III	Powder×1	2-8°C
Reagent IV	Liquid 15 mL×1	2-8°C
Reagent V	Powder×1	-20°C

Solution Preparation:

- Extract Solution:** Before use, the Extract Solution is diluted 10 times with distilled water and then used, and the diluted extract can be stored at 2-8°C for 8 weeks.
- 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°C 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.
- Reagent V:** The reagent is 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°C for 4 weeks, avoid repeated freezing and thawing.

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, adjustable pipettes, micro quartz cuvette/96 well UV plate, mortar and homogenizer, anhydrous ethanol (>98%, AR),

ammonium sulfate (AR), ice and distilled water.

Procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

- (1) Homogenize in an ice bath at a ratio of 1:5~10 tissue mass (g): Extract Solution (mL) (it is recommended to weigh about 0.2g of tissue and add 2mL of Extract Solution), centrifuge at 12000g for 15min at 4°C, 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 Extract Solution to the precipitate, dissolve it thoroughly and put this precipitate solution as a sample on ice for testing.

Note: The Extract Solution mentioned in sample processing is the Extract Solution after dilution of 10 times.

II. 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°C for more than 15min before use.
3. Add samples in micro quartz cuvette/96 well UV plate as follows.

Reagent name (μL)	Test tube(T)	Blank tube (B)
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°C 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 $\Delta A_T = A_{1T} - A_{2T}$, $\Delta A_B = A_{1B} - A_{2B}$, $\Delta A = \Delta A_T - \Delta A_B$. (Blank tubes only need to be done 1-2 times)

III. Calculatio

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.

$$\text{DFR activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times V_t \times 10^9 \div (C_{pr} \times V_s) \div T = 29.475 \times \Delta A \div C_{pr}$$

2. Sample weight:

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

$$\text{DFR activity (U/g mass)} = \Delta A \div (\epsilon \times d) \times V_t \times 10^9 \div (W \div 2 \div V_e \times V_s) \div T = 23.58 \times \Delta A \div W$$

ϵ : NADPH molar extinction coefficient, $6.22 \times 10^3 \text{ L/mol/cm}$; d : quartz cuvette optical path, 1cm; V_t : Total volume of reaction system, $220 \mu\text{L} = 0.22 \times 10^{-3} \text{ L}$; 10^9 : conversion factor, $1 \text{ mol} = 10^9 \text{ nmol}$; V_s : Volume of sample added, $40 \mu\text{L} = 0.04 \text{ mL}$; V_e : 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; C_{pr} : concentration of protein, mg/mL .

B 96 well UV plate

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

Note:

1. If $\Delta A_T > 1$ or $A_{1T} < A_{1B}$, you can dilute the sample (precipitation dissolution in pretreatment) with Extract Solution (dilution) or shorten the reaction time at 30°C . If ΔA_T 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 Solution for ice bath homogenization, follow the assay steps, measured with 96 well UV plate to calculate $\Delta A_T = 0.916 - 0.833 = 0.083$, $\Delta A_B = 0.723 - 0.700 = 0.023$. $\Delta A = \Delta A_T - \Delta A_B = 0.06$, brought to the formula:
DFR activity (U/g mass) = $23.58 \div 0.6$ (96 well UV plate optical path) $\times \Delta A \div W = 10.957 \text{ U/g mass}$

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