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CheKine™ Micro Dehydroascorbate Reductase (DHAR) Activity Assay Kit

Cat #: KTB3094 Size: 48 T/96 T

	Micro Dehydroascorbate Reductase (DHAR) Activity Assay Kit		
REF	**Cat #:** KTB3094	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues, Cells or Bacteria, Plasma, Serum		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Dehydroascorbate reductase (DHAR) is an important antioxidant enzyme in plants and a key enzyme that promotes ascorbic acid regeneration in the ascorbate-glutathione oxidation cycle. In the circulation DHAR maintain the normal metabolic level of ascorbic acid in plants through ascorbic acid, and plays an important role in protecting cellular components from oxidative damage. CheKine™ Micro Dehydroascorbate Reductase (DHAR) Activity Assay Kit can be used to detect biological samples such as plant tissues, cells or bacteria, plasma, serum or other liquid samples. In the kit, DHAR catalyzes the reduction of DHA by GSH to form AsA. The activity of DHA is calculated by measuring the reduction rate of DHA.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	50 mL	100 mL	4℃, protected from light
Reagent Ⅱ	8.75 mL	17.5 mL	4℃
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 265 nm
- 96-well UV plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

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: Prepared before use. 48 T add 1.25 mL deionized water, 96 T add 2.5 mL deionized water, fully dissolve. Inexhaustible reagents stored at 4℃ for 3 days, protected from light.

Reagent IV: Prepared before use. 48 T add 1.25 mL deionized water, 96 T add 2.5 mL deionized water, fully dissolve. Inexhaustible reagents stored at 4℃ for 3 days, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Plant Tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 30% or 300 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3002 Protein Quantification Kit (Bradford Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 265 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent II at 25℃ for 30 min.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microglass cuvette **in turn**)

Reagent	Test Well (μL)
Reagent III	20
Reagent IV	20
Reagent II	140
Sample	20

4. After rapid mixing, the absorbance values A_1 at 20 s and A_2 at 140 s at 265 nm are recorded. Finally calculate $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, the sample can be appropriately diluted with Reagent I , the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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.

A. 96-well UV plates calculation formula as below

Calculation of the DHAR activity

(1) Calculated by sample protein concentration

Unit definition: One unit of enzyme is defined as the reduction of 1 nmol AsA per milligram of protein at 25℃.

DHAR (U/mg prot)=ΔA÷ε÷d×V_{Total}×10^9÷(Cpr×V_{Sample})÷T=**184×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Unit definition: One unit of enzyme is defined as the reduction of 1 nmol AsA per milligram of protein at 25℃.

DHAR (U/g fresh weight)=ΔA÷ε÷d×V_{Total}×10^9÷(w×V_{Sample}÷$V_{Total\ Sample}$)÷T=**184×ΔA÷w**

(3) Calculated by number of cells or bacteria

Unit definition: One unit of enzyme is defined as the reduction of 1 nmol AsA per 10^4 cells or bacteria at 25℃.

DHAR(U/10^4)=ΔA÷ε÷d×V_{Total}×10^9÷(n×V_{Sample}÷$V_{Total\ Sample}$)÷T=**184×ΔA÷n**

(4) Calculated by volume of liquid samples

Unit definition: One unit of enzyme is defined as the reduction of 1 nmol AsA per milliliter liquid at 25℃.

DHAR(U/mL)=ΔA÷ε÷d×V_{Total}×10^9÷V_{Sample}÷T=**184×ΔA**

ε: TNB molar extinction coefficient at 265 nm, 5.42×10^4 L/mol /cm; 10^6: Conversion coefficient, 1 mol=10^6 μmol; d: 96-well plate optical diameter, 0.5 cm; V_{Total}: Total reaction volume, 2×10^{-4} L; V_{Sample}: Sample volume added in the reaction system, 0.02 mL; $V_{Total\ Sample}$: The volume of adding Reagent Ⅰ, 1 mL; Cpr; Sample protein concentration, mg/mL; W: Sample weight, g; T: Reaction time, 2 min; n: Total number of cells or bacteria, calculated in units of ten thousand.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Precautions

The protein concentration of the sample needs to be determined by yourself. Since Extraction Buffer contains a relatively high protein concentration (about 1 mg/mL), the protein concentration of Extraction Buffer must be deducted when measuring the protein concentration of the sample.

Typical Data

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

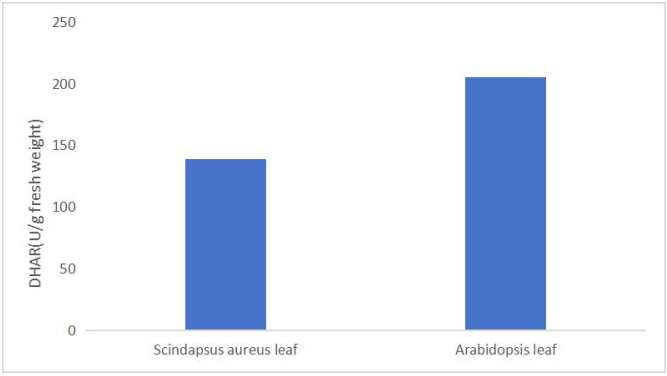

Figure 1. Determination of DHAR activity in scindapsus aureus leaf and arabidopsis leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB3090	CheKine™ Micro Dehydroascorbic Acid (DHA) Content Assay Kit
KTB3091	CheKine™ Micro Ascorbate Peroxidase (APX) Activity Assay Kit
KTB3093	CheKine™ Micro Ascorbic Acid Oxidase (AAO) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.