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CheKine™ Micro Hydroxyl Free Radical Content Assay Kit

Cat #: KTB1094 Size: 48 T/48 S 96 T/96 S

	Micro Hydroxyl Free Radical Content Assay Kit		
REF	**Cat #:** KTB1094	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Bacteria, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Hydroxyl free radical (OH^-) is a type of reactive oxygen species. CheKine™ Micro Hydroxyl Free Radical Content Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria, cells, plasma, serum or other liquid samples. In the kit, 2-deoxyribose is oxidized to a malondialdehyde analog in the presence of OH^-, and then condensed with thiobarbituric acid (TBA) to form a colored product. By measuring the maximum absorption peak of the colored product at 532 nm, the OH^- content is calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	90 mL	90 mL×2	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅳ	30 mL	30 mL×2	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 532 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, PBS
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

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

Working Reagent II: Prepared before use. Add 7.5 mL Reagent Ⅰ to each Reagent Ⅱ for 48 T, and 15 mL Reagent Ⅰ to each Reagent Ⅱ for 96 T to fully dissolve. The remaining reagent can be stored in 4℃ for 1 month, protected from light.

Working Reagent III: Prepared before use. Take one bottle of Reagent Ⅳ and transfer it all in one bottle of Reagent Ⅲ to fully dissolve. It can be sonicated until completely dissolved, the remaining reagent can be stored in 4℃ for 1 week, protected from light, and discarded if they turn pink.

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

Note: Working Reagent Ⅲ or Reagent Ⅳ has certain irritation, so personal protection is recommended during use.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for 1 month.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize or mortar on ice. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria or cells: Collect 5×10^6 bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ to ultrasonically disrupt the bacteria or cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly. If turbid, centrifuge and use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA 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 532 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Control Tube (μL)	Test Tube (μL)
Sample	60	60
Reagent Ⅰ	180	60
Working Reagent Ⅱ	0	120
Mix well, incubate for 1 h at 35℃, protected from light.		
Working Reagent Ⅲ	240	240

3. Mix well, incubate for 10 min at 95℃, take out and cool to room temperature in the ice bath. Centrifuge at 12,000 g for 10 min at 25℃. Then transfer 200 μL of each reaction to a 96-well plate or microglass cuvette, the absorbance value is measured at at 532 nm. The Control Well is marked as $A_{Control}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A=A_{Test}-A_{Control}$.

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 0.005, increase the sample quantity appropriately. If ΔA is greater than 0.4, 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.

Calculation of the OH^- content

(1) Calculated by protein concentration

Active unit definition: The absorbance value at 532 nm is changed by 0.001 per milligram of protein per h during the reaction was defined as one unit of OH^-.

OH^- (0.001 A/mg prot)=ΔA÷(Cpr×V_{Sample})÷T÷0.001**=16,667×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: The absorbance value at 532 nm is changed by 0.001 per gram tissue per h during the reaction was defined as one unit of OH^-.

OH^- (0.001 A/g fresh weight)=ΔA÷(w×V_{Sample}÷V_{Total})÷T÷0.001**=16,667×ΔA÷w**

(3) Calculated by number of bacteria or cells

Active unit definition: The absorbance value at 532 nm is changed by 0.001 per 10^4 bacteria or cells per h during the reaction was defined as one unit of OH^-.

OH^- (0.001 A/10^4)=ΔA÷(n×V_{Sample}÷V_{Total})÷T÷0.001**=16,667×ΔA÷n**

(4) Calculated by volume of liquid samples

Active unit definition: The absorbance value at 532 nm is changed by 0.001 per mL liquid sample per h during the reaction was defined as one unit of OH^-.

OH^- (0.001 A/mL)=ΔA÷V_{Sample}÷T÷0.001**=16,667×ΔA**

V_{Sample}: Added the sample volume, 0.06 mL; V_{Total}: Added Reagent Ⅰ volume, 1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; n: Number of bacteria or cells, calculated in units of ten thousand; T: Reaction time, 1 h; A: Absorbance units.

Typical Data

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

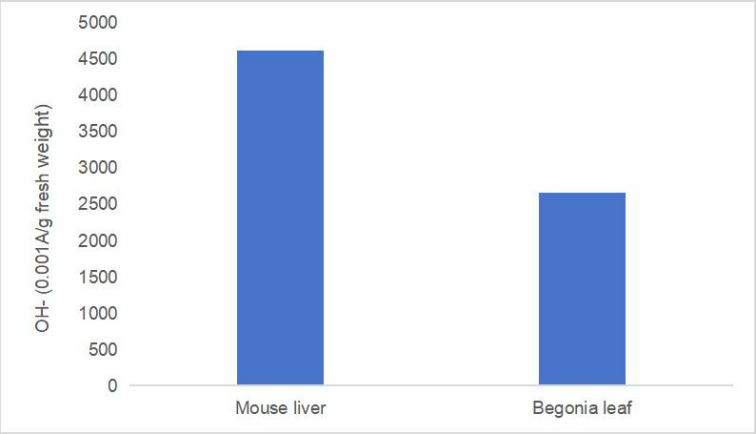

Figure 1. Determination of OH^- in mouse liver and begonia leaf by this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content 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.