Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit

Cat #: KTB1091 Size: 48 T/48 S 96 T/96 S

	Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit		
REF	**Cat #**: KTB1091	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Serum, Plasma, Cells, Bacteria, Cell Supernatant, Juice, Honey, and Urine		
	Storage: Storage at 4℃ for 12 months, protected from light		

Assay Principle

The hydroxyl radical·OH, is the neutral form of the hydroxide ion (OH^-), highly reactive (easily becoming hydroxyl groups). Hydroxyl free radicals act on biological molecules such as proteins, nucleic acids, and lipids in the body, causing damage to cell structure and function, which in turn leads to metabolic disorders in the body and causes diseases. Hydroxyl free radical scavenging capacity is one of the important indicators of the antioxidant capacity of samples, and it has been widely used in the research of antioxidant health products and drugs. CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit is specially developed for the detection of hydroxyl free radical scavenging capacity in various sample. The operation is simple and convenient, and the detection is more sensitive and accurate. In this assay, H_2O_2/ Fe^{2+} generates hydroxyl free radical through the Fenton reaction. Salicylic acid can effectively capture the generated hydroxyl free radical and reacts with them to produce 2,3-dihydroxybenzoic acid with a maximum absorption peak at 520 nm. After the substances with the capacity to scavenge hydroxyl free radical, resulting in the decrease of 520 nm absorbance. The value of 520 nm absorbance can reflect the hydroxyl free radical scavenging capacity of the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Ferrous Salt	10 mL	20 mL	4℃, protected from light
H_2O_2	5 mL	10 mL	4℃, protected from light
Salicylic Acid	10 mL	20 mL	4℃, protected from light

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 520 nm
- Incubator, ice maker, refrigerated centrifuge

- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Ferrous Salt: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
H_2O_2: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Salicylic Acid: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Note: Ferrous Salt is corrosive, Salicylic Acid is irritating to the skin and mucous membranes, and personal protection is recommended when using.

Sample Preparation

1. Animal tissue: Weigh 0.1 g tissue, add 1 mL deionized water and homogenize in ice bath. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay.
2. Plant tissue: Weigh 0.1 g tissue, add 1 mL deionized water and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay.
3. Cell or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cell or bacterium with cold PBS, discard the supernatant after centrifugation; add 1 mL deionized water to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay.
4. Plasma, serum and other biological fluids with high protein content or turbidity: Take 0.1 mL sample, add 1 mL deionized water and mix well. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay.
5. Juice, honey, urine and other biological fluids with low protein content and clear: Tested directly.
6. Extract or drug: Formulated to a certain concentration, such as 0.5 mg/mL to test.

Assay Procedure

1. Preheated the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each well:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control Well (μL)
Ferrous Salt	40	40	40	40
H_2O_2	0	40	40	0
Deionized Water	120	80	40	80
Salicylic Acid	40	40	40	40
Sample	0	0	40	40

Mix well, then incubate 20 min in 37℃. And reading the values at 520 nm. Finally, calculate $\Delta A_{Test} = A_{Test} - A_{Control}$, $\Delta A_{Standard} = A_{Standard} - A_{Blank}$.

Note: (1) In order to compare the hydroxyl free radical scavenging capacity of different samples, the same batch of samples must be diluted by the same multiple, and the extracts or drugs must be formulated to the same concentration. (2) The Blank Well and the Standard Well only need to be done 1-2 times. Each Test Well needs to have a Control Well. (3) If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is larger than $\Delta A_{Standard}$, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Calculation formula:

Hydroxyl free radical scavenging rate D%=($\Delta A_{Standard}$-ΔA_{Test})÷$\Delta A_{Standard}$×100%

Typical Data

Examples

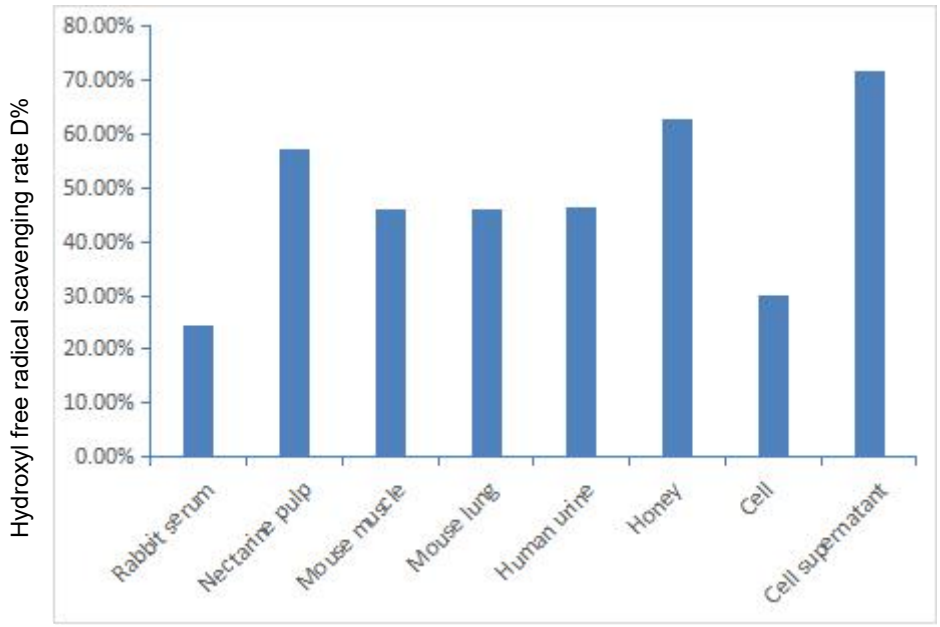

Figure 1. Hydroxyl free radical scavenging rate D% in Rabbit serum, Nectarine pulp, Mouse muscle, Mouse lung, Human urine, Honey, Cell, and Cell supernatant respectively. Assays were performed following kit protocol.

Precautions

1. Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if used separately or substituted.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Avoid cross-contamination, change pipette tips between additions of standards, samples and reagents. Also, use separate reservoirs for each reagent.
4. Ensure all reagents and equipment are at the appropriate temperature before starting the assay.
5. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If the sample value is higher than the standard value or lower than the blank value, please further dilute the sample with deionized water.
6. Observe good laboratory practices. Gloves and lab coat should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1520	CheKine™ Micro Plant Oligomeric Proantho Cyanidins (OPC) Assay Kit
KTB1530	CheKine™ Micro Plant Flavonoids Assay Kit

KTB1540	CheKine™ Micro Plant Total Phenols (TP) Assay Kit
KTB1550	CheKine™ Micro Ceruloplasmin (Cp) Activity 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.