Hydroxyl Free Radical Assay Kit

Cat. No. Kit-0889 **Lot. No.** (See product label)

SPECIFICATION

Description	Fenton Reaction is the most common chemical reaction that generates hydroxyl free radical. The amount of H2O2 is proportional to the amount of OH^- generated in Fenton reaction. When the electron acceptor is provided, the color develops with Griess reagent and form red substance. The color development is proportional to the amount of OH^-.
Notes	1) Each tube must be done separately, and reaction time must be one minute precisely. 2) Reagents must be added strictly in accordance with the order of operating table. Don't prepare mixed reagents. 3) The sample solvent or medium can be normal saline and double distilled water, but must not be phosphate buffer solution or absolute ethanol, etc.. 4) This method has a high detection sensitivity. When detecting samples except serum (plasma) and tissue, you'd better take the stock solution and diluted samples at different concentrations for the pre-test, such as 1:5 dilution solution or 1:10 dilution solution. If the color of the testing tube is too light, the sample may be diluted with its solvent until the color is darker. Hydroxyl free radical in water extract of pollen that has been tested showed a clearer coloration when stock solution is diluted 150 times.
Size	50 Tubes/48 Samples
Kit Components	Reagent 1: 3% H2O2 standard stock solution, 0.5 mL× 1 vial, can be stored at 4°C for 3 months. Preparation of 0.03% standard working solution: double distilled water = 1:99, diluted

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before use.
Reagent 2: substrate stock solution, 1 mL × 1 vial, can be stored at 4°C for 3 months.
Preparation of substrate working solution:
If your sample is to inhibit hydroxyl free radical, which means the absorbance of the testing tube is lower than that of the contrast tube, substrate working solution should be prepared as substrate stock solution : double distilled water =1:99 before use.
If your sample is to generate hydroxyl free radical, which means the absorbance of the testing tube is higher than that of the contrast tube, substrate working solution should be prepared as substrate stock solution : double distilled water =1:299 before use.
Reagent 3:
A stock solution, 2 mL × 1 vial, can be stored at 4°C for 3 months. Add double distilled water at the ratio of 9:1 before use.
B stock solution, 7 mL × 2 vials, can be stored at 4°C for 3 months.
Preparation of R3 working solution: Add the same volume of working solution A into B to give required solution. Store the remaining at 4°C for 3 months.
Reagent 4: Liquid, 10 mL × 1 vial, can be stored at 4°C for 3 months. Add double distilled water until the solution reach 100 mL before use, stored at 4°C. The liquid should be placed in water bath at 37°C if crystallization occurs. Dissolve the crystal before dilution.
Reagent 5: Liquid, 30 mL ×1 vial, can be stored at 4°C for 3 months when protected from the light.
Reagent 6: Liquid, 30 mL ×1 vial, can be stored at 4°C for 3 months when protected from the light.
Reagent 7: Analytically pure glacial acetic acid should be self-provided.
Preparation of chromogenic agent: Working solution R4: R5: R6: glacial acetic acid = 8:3:3:2, prepare fresh solution before use.
Notes:
There are substances that inhibit hydroxyl free radical such as serum (plasma), various tissue homogenate and oral liquid, etc.; there are substances that generate

	hydroxyl free radical such as neutrophils, certain drugs and some plants, etc..
Assay Protocol	Working solutions as described above should be preheated in water bath at 37°C for 3 min. Procedures indicated below should be carried out in water bath at 37°C. Blank Standard Contrast Sample Double Distilled Water(mL) 0.4 0.2 0.2 H2O2 Standard Working 0.2 Solution(mL) Substrate Working 0.2 0.2 Solution(mL) Sample*(mL) 0.2 R3 Working Solution(mL) 0.4 0.4 0.4 0.4 Mix fully, reacting at 37°C for 1 minute (time with a stopwatch for accuracy). Add the Chromogenic agent immediately 1 minute after the addition of R3 to terminate the reaction. Only one tube at a time. Chromogenic Agent(mL) 2 2 2 2 Mix fully, place at room temperature for 20 minutes; wavelength: 550nm, optical path: 1cm; set to zero with double distilled water and determinate the absorbance value of each tube. *Reference sampling amount: Plasma (serum) specimen is diluted with normal saline in the ratio of 1:20. Then 0.2 mLsample is drawn for the measurement. If you have an accurate micropipette, you can take 0.010 mL plasma (serum) directly, and add 0.190 mL normal saline. 0.2 mL Supernatant of tissue homogenate is drawn for the measurement. Preliminary test needs to be done to determine specific sampling amount.
Analysis	1) The inhibitory capacity to hydroxyl free radical in plasma (serum) is calculated as follows: Definition Per milliliter plasma (serum) react for 1 minute at 37°C to reduce the concentration of H2O2 in the reaction system by 1 μmol/mL is a unit of inhibitory

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capacity to hydroxyl free radical.

Formula: Inhibitory capacity to hydroxyl free radical (U/mL) = (ODContrast−ODSample)/ (ODStandard−ODBlank) × Concentration of standard (8.824 umol/ml) × (1 ml/Sampling volume) × Dilution factor of sample before tested

Examples: Plasma (serum) specimen was diluted with normal saline in the ratio of 1:20. Then 0.2 mL sample was drawn to measure the inhibitory capacity of hydroxyl free radical. The OD of each tube are as following: ODContrast is 0.785, ODSample is 0.464, ODStandard is 0.443, ODBlank is 0.003. And the standard concentration of H_2O_2 was 8.824μmol/mL, as shown below:

Inhibitory capacity to hydroxyl free radical (U/mL) = (0.785−0.464)/ (0.443−0.003) × 8.824) × (1/ 0.2) × 20 =643.75 U/mL

2) The inhibitory capacity to hydroxyl free radical in tissues is calculated as follows:

Definition: Per milligram tissue proteins react for 1 minute at 37°C to reduce the concentration of H2O2 in the reaction system by 1μmol/mL is a unit of inhibitory capacity to hydroxyl free radical

Formula: Inhibitory capacity to hydroxyl free radical (U/mgprot) =(ODContrast−ODSample)/ (ODStandard−ODBlank) × Concentration of standard (8.824 umol/ml) ÷ [Protein concentration of tested sample(mgprot/ml) × Sampling volume (0.2 mL)]

Examples: 5% mouse liver homogenate was diluted with normal saline in the ratio of 1:20 to 0.5% liver homogenate. Then 0.2 mL of it was drawn to measure the inhibitory capacity of hydroxyl free radical. The OD of each tube are as following: ODContrast is 0.785, ODSample is 0.347, ODStandard is 0.443, ODBlank is 0.003. The standard concentration of H_2O_2 was 8.824 μmol/mL and the protein of 0.5% mouse liver homogenate was 0.485 mg/mL, as shown below:

Inhibitory capacity to hydroxyl free radical (U/mgprot) = (0.785−0.347)/ (0.443−0.003) × 8.824 ÷ (0.486 × 0.2) =90.37 U/mgprot

3) The inhibitory capacity to hydroxyl free radical in hemolysis fluid is calculated as follows:

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Definition: Per milligram hemoglobin should react for 1 minute at 37°C to reduce the concentration of H2O2 in the reaction system by 1 μmol/mL is a unit of inhibitory capacity to hydroxyl free radical.

Formula: Inhibitory capacity to hydroxyl free radical(U/mgHb)= (ODContrast−ODSample)/ (ODStandard−ODBlank) × Concentration of standard (8.824 umol/ml) × (1 ml/Sampling volume) × Dilution factor of sample before tested ÷ Concentration of hemoglobin (mgHb/mL) Examples:

A. Add 0.8 mL double distilled water to 0.2 mL anticoagulant erythrocytes, then mix well for 1 minute in whirlpool mixer to prepare hemolysis fluid. The hemoglobin was determined as 41.182 mgHb/mL. Add 5.99 mL double distilled water to 0.01 mL hemolysis fluid and mix well. Then take 0.2 mL mixed solution for the measurement in accordance to the operation table. The OD of each tube are as following: ODContrast is 0.785, ODSample is 0.615, ODStandard is 0.443, ODBlank is 0.003.The standard concentration of H_2O_2 was 8.824 μmol/mL, as shown below:

Inhibitory capacity to hydroxyl free radical (U/mgHb) = (0.785−0.615)/(0.443−0.003) × 8.824 × (1/ 0.2) × 600÷ 41.182= 248.36 U/mgHb

B. Add 0.8 mL double distilled water to 0.2 mL anticoagulant erythrocytes, then mix well for 1 minute in whirlpool mixer to prepare hemolysis fluid. And the hemoglobin was determined to be 53.684 mg Hb/mL. Add 5.99 mL double distilled water to 0.01 mL hemolysis fluid and mix well. Then 0.2 mL of it is drawn for the measurement in accordance to the operation table. The OD of each tube are as following: ODContrast is 0.785, ODSample is 0.304, ODStandard is 0.443, ODBlank is 0.003. And the standard concentration of H_2O_2 was 8.824 μmol/mL, as shown below:

Inhibitory capacity to hydroxyl free radical(U/mL)= (0.785−0.304)/ (0.443−0.003) × 8.824 ×(1/ 0.2) × 600÷ 53.684= 539.06 U/mgHb

4) The capacity to generate hydroxyl free radical is calculated as follows:

Definition: The concentration of H2O2 in the reaction solution was reduced by 1μmol/mL is a unit of inhibitory capacity to hydroxyl free radical in the reaction system in 106 cells per milliliter or per milligram of substance or per cubic centimeter.

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Formula: Productive capacity to hydroxyl free radical(U/mL)= (ODSample−ODContrast)/ (ODStandard−ODBlank) × Concentration of standard (8.824 umol/ml) × (1 ml/Sampling volume) × Dilution factor of sample before tested

Examples: Some traditional Chinese medicine (TCM) was diluted 10 times, from which 0.2 mL was drawn for the measurement. The OD of each tube are as following: ODContrast is 0.217, ODSample is 0.621, ODStandard is 0.443, ODBlank is 0.003, as shown below:

Productive capacity to hydroxyl free radical(U/mL)= (0.621−0.217)/(0.443−0.003)× 8.824 × (1/0.2)× 10 = 405.10 U/mL

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