

Hydroxy Radical Assay kit

(Cat.No: BC000 Size:50T/48S)

1. Assay principle (Spectrophotometric method)

Fenton reaction is the most common hydroxy radical producing chemical reaction, H_2O_2 amount appears direct proportion with $OH^\cdot$ amount produced by Fenton reaction. It receives electron acceptor and reacts with Griess reagent to produce red compound, its absorbance appears direct proportion with $OH^\cdot$ amount.

This kit can be used for laboratory research only.

2. Components of the Kit (The kit is valid for 3 months)

		Specification	Storage
Reagent 1	3% H_2O_2 standard stock solution Liquid	0.5 mL × 1 bottle	Stored at 4 °C for 3 months
Preparation of 0.03% standard working solution: Dilute 3% H_2O_2 standard stock solution with double-distilled water at a ratio of 1:99. Prepare freshly before use.			
Reagent 2	Substrate stock solution	1 mL × 1 bottle	Stored at 4 °C for 3 months
Preparation of substrate working solution: - If your sample inhibits hydroxyl radicals, i.e., the absorbance of the test tube is lower than that of the control tube, prepare the substrate working solution as follows: substrate stock solution : double-distilled water = 1:99, prepare freshly. - If your sample generates hydroxyl radicals, i.e., the absorbance of the test tube is higher than that of the control tube, prepare the substrate working solution as follows: substrate stock solution : double-distilled water = 1:299, prepare freshly.			
Reagent 3	Solution A stock solution	2 mL × 1 bottle	Stored at 4 °C for 3 months
	Solution B	7 mL × 2 bottles	Stored at 4 °C for 3 months
Solution A working solution: dilute Solution A with double-distilled water at a ratio of 1:9 before use. Preparation of Reagent 3 working solution: mix Solution A working solution and Solution B at equal volumes. Prepare according to need. The remaining solution can be stored at 4 °C for one week.			
Reagent 4	Liquid	10 mL × 1 bottle	Stored at 4 °C for 3 months
Reagent 4 working solution: dilute with double-distilled water to 100 mL before use to prepare the working solution, store at 4 °C. If crystals appear, place in a 37 °C water bath until completely dissolved before dilution.			
Reagent 5	Liquid	30 mL × 1 bottle	Store at 4 °C protected from light for 3 months



Reagent 6	Liquid	30 mL × 1 bottle	Store at 4 °C protected from light for 3 months
Reagent 7	Glacial acetic acid (analytical grade), user-provided.		
Preparation of chromogenic reagent: Reagent 4 working solution : Reagent 5 : Reagent 6 : glacial acetic acid = 8 : 3 : 3 : 2, prepare freshly before use.			

[Note]:

Substances that inhibit hydroxyl radicals include serum (plasma), various tissue homogenates, oral liquids, etc.;

Substances that generate hydroxyl radicals include neutrophils, certain drugs, and some plants.

3. Operation Procedure

Pre-warm all prepared working solutions in a 37 °C water bath for 3 minutes. Perform the following steps in a 37 °C water bath.

	Blank tube	Standard tube	Control Tube	Test Tube
Double-distilled water (mL)	0.4	0.2	0.2	
0.03% H ₂ O ₂ standard working solution (mL)		0.2		
Substrate working solution (mL)			0.2	0.2
Sample* (mL)				0.2
Reagent 3 working solution (mL)	0.4	0.4	0.4	0.4
Start timing immediately when adding Reagent 3, mix rapidly, react at 37 °C for 1 minute (timed accurately with a stopwatch), then immediately add chromogenic reagent to terminate the reaction.				
Chromogenic reagent (mL)	2	2	2	2
Mix well, stand at room temperature for 20 minutes, measure absorbance (A) at 550 nm with a 1 cm optical path length, zero with double-distilled water.				

*Sampling reference:

Serum (plasma) samples are diluted 20-fold with normal saline, then 0.2 mL is taken for detection; if a precise micropipette is available, directly take 10 µL serum (plasma) and add 190 µL normal saline.

For 10% tissue homogenate supernatant, take 0.2 mL for detection.

The specific sampling concentration should be determined according to pre-test results. See the appendix for detailed pre-test methods.

4. Calculation and Examples

(1) Calculation of hydroxyl radical inhibition capacity in serum (plasma):

① Definition: One unit of hydroxyl radical inhibition capacity is defined as the amount



per milliliter of serum (plasma) that reduces the H_2O_2 concentration in the reaction system by 1 mmol/L after reacting for 1 minute at 37 °C.

② Formula:

$$\text{Hydroxylradical inhibition capacity (U/ml)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times \frac{1}{V_{\text{sample}}} \times \text{Sample dilution factor before test (N - fold)}$$

C_{standard} : standard concentration, 8.824 mmol/L;

V_{sample} : sample volume, 0.2 mL;

N: dilution factor before sample testing.

(2) Calculation of hydroxyl radical inhibition capacity in tissues:

① Definition: One unit of hydroxyl radical inhibition capacity is defined as the amount per milligram of tissue protein that reduces the H_2O_2 concentration in the reaction system by 1 mmol/L after reacting for 1 minute at 37 °C.

② Formula:

$$\text{Hydroxylradical inhibition capacity (U/mgprot)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times (C_{\text{pr}} \times V_{\text{sample}}) \times \text{Sample dilution factor before test (N - fold)}$$

C_{standard} : standard concentration, 8.824 mmol/L;

V_{sample} : sample volume, 0.2 mL;

C_{pr} : protein concentration of tissue homogenate, mgprot/mL (prot refers to protein);

N: dilution factor before sample testing.

(3) Calculation of hydroxyl radical inhibition capacity in hemolysate:

① Definition: One unit of hydroxyl radical inhibition capacity is defined as the amount per milligram of hemoglobin that reduces the H_2O_2 concentration in the reaction system by 1 mmol/L after reacting for 1 minute at 37 °C.

② Formula:

$$\text{Hydroxylradical inhibition capacity (U/ml)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times \frac{1}{V_{\text{sample}}} \times \text{Sample dilution factor before test (N - fold)} \div \text{CHb}$$

C_{standard} : standard concentration, 8.824 mmol/L;

V_{sample} : sample volume, 0.2 mL;

CHb: hemoglobin concentration in hemolysate, mgHb/mL (Hb refers to hemoglobin);

N: dilution factor before sample testing.

(4) Calculation of hydroxyl radical generation capacity:

① Definition: One unit of hydroxyl radical generation capacity is defined as the amount per milliliter, per milligram of substance, or per 10^6 cells per cubic centimeter that increases the H_2O_2 concentration in the reaction system by 1 mmol/L.

② Formula:



$$\text{Hydroxylradical generation capacity (U/ml)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{standard}} - A_{\text{blank}}} \times C_{\text{standard}} \times \frac{1}{V_{\text{sample}}} \times \frac{\text{Sample dilution factor before test (N - fold)}}{1}$$

C_{standard} : standard concentration, 8.824 mmol/L;

V_{sample} : sample volume, 0.2 mL;

N: dilution factor before sample testing.

5. Precautions

1. The reaction time for each tube (from adding Reagent 3 to adding chromogenic reagent) must be exactly 1 minute.
2. Reagents must be added strictly according to the operation table order; mixed reagents must not be prepared.
3. The solvent or medium for test samples may be normal saline, double-distilled water, or acetic acid, but **not phosphate buffer or absolute ethanol**.
4. This method has high sensitivity. When testing samples other than serum (plasma) and tissues, it is recommended to perform pre-tests using the original solution and different dilutions (e.g., 5-fold or 10-fold dilutions). If the color of the test tube is too light, further dilute the sample with its solvent until sufficient color develops. For hydroxyl radical detection in pollen water extracts, diluting the original solution 150-fold gives better color development.

Appendix I: Determination of optimal sample concentration and optimal sample volume for hydroxyl radical assay

I. Sample pretreatment:

1. Serum (plasma): Dilute serum (plasma) with normal saline at ratios of 1:1, 1:4, 1:9, 1:19, etc., to prepare a series of different concentrations. Take 0.2 mL of each dilution and test according to the serum (plasma) operation table.

2. Tissue homogenates, cells, mitochondria, or cell membranes: Dilute tissue homogenates with normal saline to prepare a series of concentrations such as 10%, 5%, 2%, 1%, 0.5%, and 0.1%. Take 0.2 mL of each concentration and test according to the tissue operation table.

II. Operation procedure (example for plasma samples to determine optimal sampling concentration):

1. Sample source: Whole blood collected from the orbital sinus of normal rats, anticoagulated with heparin, plasma obtained for testing.

2. Sample dilution: Dilute plasma with normal saline at ratios of 1:1, 1:4, 1:9, 1:19, 1:49, and 1:99 to prepare a series of different concentrations. Take 0.2 mL of each dilution and test according to the serum (plasma) operation table.

3. Operation table:

	Blank Tube	Standard Tube	Control Tube	Test Tube
Double-distilled water (mL)	0.4	0.2	0.2	
0.03% H ₂ O ₂ standard working solution (mL)		0.2		
Reagent 2 (substrate working solution) (mL)			0.2	0.2
Sample* (mL)				0.2
Reagent 3 (mL)	0.4	0.4	0.4	0.4
Start timing immediately when adding Reagent 3, mix rapidly, react at 37 °C for 1 minute (timed accurately with a stopwatch), then immediately add chromogenic reagent to terminate the reaction.				
Chromogenic reagent (mL)	2	2	2	2
Mix well, stand at room temperature for 20 minutes, measure absorbance at 550 nm with a 1 cm optical path length, zero with double-distilled water.				

4. Pre-test results:

Control	0.785		Blank	0.003	Standard	0.443
Dilution ratio	1:1	1:4	1:9	1:19	1:49	1:99
Absorbance	0.105	0.132	0.188	0.408	0.677	0.741
Inhibition rate	86.59%	83.16%	76.01%	47.99%	13.71%	5.66%



5. Conclusion:

From the above data, the optimal sampling concentration is when the inhibition rate

($\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%$) is between 45% and 55%, which corresponds to a dilution of 1:19.

Therefore, 0.2 mL of 1:19 diluted normal rat plasma is recommended for determination.

III. Important notice:

Before formal testing, 2–3 samples from each group should be used for the above pre-test to determine the optimal sample concentration or optimal sample volume. While ensuring that the

inhibition rate ($\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%$) is between 20% and 55%, there should be differences

between groups. If not, re-determination of the optimal sample concentration or sample volume is required.