PRODUCT INFORMATION & MANUAL

Hydroxyl Free Radical Assay Kit (Colorimetric)

NBP3-25831

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Hydroxyl Free Radical Assay Kit (Colorimetric)

Catalog No: NBP3-25831
Method: Colorimetric method
Specification: 50Assays **(**Can detect 48 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 10.1U/mL
Detection range: 10.1-736.7U/mL

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can measure Hydroxyl Free Radical (-OH) content in serum, plasma, tissue, hemolysate and other samples.

Detection principle

Fenton Reaction is the most common chemical reaction that generates hydroxyl free radical. The amount of H_2O_2 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^-.

Kit components

	Components	**Specifications**	**Storage**
Reagent 1	3% H_2O_2 Standard Stock Solution	0.5 mL × 1 vial	2-8℃, 12 months
Preparation of 0.03% H_2O_2 standard solution: dilute the reagent 1 with double distilled water at a ratio of 1:99. Prepare the fresh solution before use.			
Reagent 2	Substrate Stock Solution	1 mL × 1 vial	2-8℃, 12 months
Preparation of substrate working solution (prepare the fresh solution before use)**:** a) If hydroxyl free radical of sample is inhibited, which means the absorbance of the sample tube is lower than that of the control tube, substrate working solution should be prepared at a ratio of substrate stock solution: double distilled water =1:99. b) If your sample is to generate hydroxyl free radical, which means the absorbance of the sample tube is higher than that of the control tube, substrate working solution should be prepared at a ratio of substrate stock solution : double distilled water =1:299. **Notes:** Samples that inhibit hydroxyl free radical such as serum, plasma, tissue homogenate and oral liquid, etc. Samples that generate hydroxyl free radical such as neutrophils, certain drugs and some plants, etc.			
Reagent 3 (A)	Stock Solution A	2 mL × 1 vial	2-8℃, 12 months
Preparation of reagent 3 working solution: dilute the reagent 3 with double distilled water at a ratio of 1:9 and mix fully.			
Reagent 3 (B)	Solution B	7 mL × 2 vials	2-8℃, 12 months
Preparation of reagent 3 application solution: mix reagent 3 working solution and reagent 3 (B) at a ratio of 1:1. Prepare the needed amount before use.			
Reagent 4	Liquid	10 mL × 1 vial	2-8℃, 12 months
Preparation of reagent 4 application solution: dilute the reagent 4 with double distilled water to a final volume of 100 mL and store at 2-8℃. [Note]: The liquid should be incubate at 37℃ water bath if crystal appears. Dissolve the crystal before dilution.			

Reagent 5	Liquid	30 mL ×1 vial	2-8 ℃, 12 months, shading light
Reagent 6	Liquid	30 mL ×1 vial	2-8 ℃, 12 months, shading light
Preparation of chromogenic agent: mix the reagent 4 application solution, reagent 5, reagent 6 and glacial acetic acid (self-prepared) at a ratio of the reagent 4 application solution: reagent 5: reagent 6: glacial acetic acid=8:3:3:2. Prepare the fresh solution before use.			

Operation steps

Prepared solutions as described above should be preheated in water bath at 37℃ for 3 min. Procedures indicated below should be carried out in 37℃ water bath.

	Blank tube	Standard tube	Control tube	Sample tube
Double distilled water (mL)	0.4	0.2	0.2	
0.03% H_2O_2 standard solution (mL)		0.2		
Substrate working solution (mL)			0.2	0.2
Sample* (mL)				0.2
Reagent 3 application solution (mL)	0.4	0.4	0.4	0.4
Mix fully, react at 37℃ for 1 min accurately. Add the chromogenic agent immediately to stop the reaction. (Note: It is suggested to carry the assay with a tube only at a time.)				
Chromogenic agent (mL)	2	2	2	2
Mix fully, stand at room temperature for 20 min. Set spectrophotometer to zero with double distilled water and measure the OD value of each tube at 550 nm with 1 cm optical path cuvette.				

***Reference sampling volume:**

Plasma (serum) sample: dilute the sample with normal saline for 20 times and take 0.2 mL for detection. Tissue homogenate sample: take 0.2 mL for detection. It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment.

Calculation of results

1. **The inhibitory capacity to hydroxyl free radical in plasma (serum) sample:**

   Definition: The ability of hydroxyl free radical (-OH) in 1 mL of serum or plasma that reduce 1 μmol/L H_2O_2 in the reaction system (1 min, 37℃) is defined as a unit of inhibitory capacity to hydroxyl free radical.

   Inhibitory capacity to hydroxyl free radical (*U/mL*)

   $= \frac{OD_{Control} - OD_{Sample}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard $(8.824\ mmol/mL)$

   $\times \frac{1\ mL}{\text{Sampling volume}} \times$ Dilution factor of sample before tested

2. **The inhibitory capacity to hydroxyl free radical in tissues:**

   Definition: The ability of hydroxyl free radical (-OH) in 1 mg of tissue protein that reduce 1 μmol/L H_2O_2 in the reaction system (1 min, 37℃) is defined as a unit of inhibitory capacity to hydroxyl free radical.

   Inhibitory capacity to hydroxyl free radical (*U/mgprot*)

   $= \frac{OD_{Control} - OD_{Sample}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard $(8.824\ \mu mol/mL)$

   $\div$ [Protein concentration of tested sample$(mgprot/mL) \times$ Sampling volume $(0.2\ mL)$]

3. **The inhibitory capacity to hydroxyl free radical in hemolysate:**

   Definition: The ability of hydroxyl free radical (-OH) in 1 mg of hemoglobin that reduce 1 μmol/L H_2O_2 in the reaction system (1 min, 37℃) is defined as a unit of inhibitory capacity to hydroxyl free radical.

   Inhibitory capacity to hydroxyl free radical (*U/mgHb*）

   $= \frac{OD_{Control} - OD_{Sample}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard $(8.824\ \mu mol/mL)$

   $\times \frac{1\ mL}{\text{Sampling volume}} \times$ Dilution factor of sample before tested

   $\div$ Concentration of hemoglobin$(mgHb/mL)$

4. **The capacity to generate hydroxyl free radical:**

   Definition: The ability of hydroxyl free radical (-OH) in 1 mL of sample or 1 mg sample or10^6 cells that increase 1 μmol/L H_2O_2 in the reaction system (1 min, 37℃) is defined as a unit of the capacity to generate hydroxyl free radical.

   Productive capacity to hydroxyl free radical (*U/mL*)

   $= \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard $(8.824\ \mu mol/mL)$

   $\times \frac{1\ mL}{\text{Sampling volume}} \times$ Dilution factor of sample before tested

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. Each tube must be done separately, and reaction time must be one minute precisely.
6. The solvent or medium for sample can be normal saline or double distilled water, not phosphate buffer solution or absolute ethanol.
7. This method has a high detection sensitivity. When detecting samples except serum (plasma) and tissue, it is recommended to take the samples (diluted or not-diluted) at different concentrations for the pre-experiment. If the color of the sample tube is too light, the sample can be diluted until the color is darker. Hydroxyl free radical in water extract of pollen that has been tested, it shows a good colour development when the sample is diluted for 150 times.