HYDROGEN PEROXIDE ASSAY

Colorimetric Method **50 Tests**
For research only

INTRODUCTION :

Hydrogen peroxide is a powerful oxidant at the center of many redox pathways. It is also a key player in the healing process as neutrophils gather at a wound site post trauma and release bactericidal reactive oxygen species (ROS) and H_2O_2 to kill bacteria and prevent infection. The ongoing interest in understanding the role of hydrogen peroxide in biological systems including its role as a second messenger prompts the need for a rapid and quantitative measurement tool. Researchers also require a method of hydrogen peroxide measurement for laboratory reagents and detergents in order to prevent the unintended contribution of peroxidation by reagent use .

PRINCIPLE :

In the presence of peroxidase (HRP), H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzensulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore

$$2\,H_2O_2 + DHBS + AAP \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H_2O$$

REAGENTS :

1.	Standard H_2O_2 (dil. 1000 times before use)	500 µM / L
2.	Chromogen Phosphate buffer pH 7.0 3,5 Dichloro-2-hydroxy benzene sulphonate Detergent	 100 mM / L 1 mM / L
3.	Enzyme 4 – Aminoantipyrine Peroxidase Preservative	 2 mM / L > 2000 U / L

PROCEDURE :

Dilute R1 1000 times immediately before use (10 ul R1 + 10 ml d. water, mix), discard after use .

	Sample ml	Standard ml	Blank ml
Sample	0.05	-	0.05
Standard (R1)	-	0.05	-
Chromogen (R2)	0.5	0.5	0.5
Enzyme (R3)	0.5	0.5	-
D. Water	-	-	0.5

Incubate 10 min. at 37 ºC. Read the sample (A_{Sample}) and standard ($A_{Standard}$) against blank at 510 nm (500 – 520 nm) .Color stable for one hour . Linearity up to 1.5 mM / L

CALCULATION :

H_2O_2 concentration :

In Plasma (mM / L) $= \dfrac{A_{Sample}}{A_{standard}} \times 0.5$

In tissue (mM / g . tissue) =

$$\frac{A_{Sample}}{A_{standard}} \times 0.5 \times \frac{1}{\text{g. tissue used}}$$

REFERENCE:

Aebi, H. (1984) Methods Enzymol 105, 121 – 126
Fossati, P., et al . (1980) Clin. Chem. 26, 227 – 231 .

SAMPLE PREPARATION

Tissue Homogenate

1. Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4 . containing 0.16 mg / ml heparin to remove any red blood cells and clots .
2. Homogenize the tissue in 5 – 10 ml cold buffer (i , e , 50 mM potassium phosphate, pH 7.5. 1 mM EDTA) per gram tissue.
3. Centrifuge at 4,000 rpm for 15 minutes at 4 °C .
4. Remove the supernatant for assay and store on ice. If not assaying on the same day , freeze the sample at - 80°C. The sample will be stable for at least one month.

Plasma

1. Collect blood using an anticoagulant such as heparin, citrate , or EDTA .
2. Centrifuge at 4,000 rpm for 10 minutes at 4°C .
3. Collect the plasma for assaying and store on ice. If not assaying on the same day , freeze at - 80°C.

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

HYDROGEN PEROXIDE

Colorimetric Method	
+4 to +8ºC	50 Tests
CAT. NO.	HP 25

REAGENTS

R1	Standard	2.5 ml
R2	Chromogen	25 ml
R3	Enzyme	25 ml

CONTACTS

Tele: 02-33385184
Mobil: 0109 – 349 20 77
Fax : 02-33385184 (102)
e.maile : info@bio-diagnostic.com
Website: www.bio-diagnostic.com
Adress: 29 Tahreer St., Dokki, Giza, Egypt