

HYDROGEN PEROXIDE ASSAY

Colorimetric Method
For research only

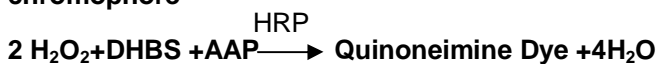
50 Tests

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 H₂O₂ 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), H₂O₂ reacts with 3,5-dichloro-2-hydroxybenzenesulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore



REAGENTS :

| | | |
|----|---|--------------------------|
| 1. | Standard H ₂ O 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 °C. 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 :

H₂O₂ concentration :

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

In tissue (mM / g . tissue) =

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

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.

HYDROGEN PEROXIDE

| | | |
|------------|---------------------|----------|
| | Colorimetric Method | |
| +4 to +8°C | | 50 Tests |
| CAT. NO. | | HP 25 |

REAGENTS

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

CONTACTS

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