



## Hydrogen Peroxide Assay Kit

- Sensitive, Quantitative Assay for Hydrogen Peroxide
- Detects as Little as 15ng/ml
- Easy-to-use, Colorimetric System

### Introduction

National Diagnostics Hydrogen Peroxide Assay Kit is a rapid, sensitive and quantitative method for the determination of Hydrogen Peroxide in chemical or biological systems. The assay is based upon formation of a complex between Xylenol Orange and ferric iron, which is produced by the peroxide dependent oxidation of ferrous iron. This reaction is quantified colorimetrically, detecting as little as 15ng/ml of peroxide. Each kit provides reagents sufficient for 100 assays.

### Assay Reagent Preparation

Prepare 1.8ml of reagent per sample to run tests in duplicate.

1. To prepare 20ml: Combine 19.8ml of Component A with 0.2ml Component B.
2. The mixture is stable at room temperature for one working day.

### Assay Procedure

1. Mix 0.9ml of Assay Reagent with up to 0.1ml of sample.
2. Incubate at room temperature for at least 30 minutes to allow for complete color development.
3. Read absorbance at 560nm.

### Frequently Asked Questions

#### Will the peroxide detection kit also detect organic peroxides (ROOH)?

Yes, color will be developed by organic peroxides, as they will also oxidize iron. In order to be sure that the signal is due to  $H_2O_2$ , a control should be run in the presence of catalase, which will eliminate the  $H_2O_2$  signal. Any absorbance which is not eliminated by catalase is not due to  $H_2O_2$ .

#### I want to do absolute quantitation of Hydrogen Peroxide - what do I use as a standard?

Diluted hydrogen peroxide may be used as a standard, provided it is standardized by UV absorption measurement prior to dilution. Hydrogen peroxide solutions can be unstable, so standardization is essential for accurate results. To determine the concentration of a Hydrogen Peroxide solution, measure the absorbance at 240nm and use a molar extinction coefficient of  $43.6M^{-1}cm^{-1}$  (JBC 245, pp2409-13, 1970) A standard 3% solution is 0.88M, and at a 1:100 dilution would have an A240 of 0.388.

#### What components of my system might interfere with the Peroxide Assay?

Chelating agents such as EDTA, radical scavengers like ethanol, DMSO or BHT, or sulfonated buffers like MES or HEPES will interfere with color development. Common components such as SDS, Phosphate, Tris, Glucose, Mannitol, Glutathione or BSA do not interfere with the assay in moderate concentrations.

### Hydrogen Peroxide Assay Kit

CL-204

100 Assay Kit