# Technical Manual

# ChromaDazzle Peroxide Assay Kit

## Catalogue Code: BA0047

## Pack Size: 250 assays

## Research Use Only

**DESCRIPTION**

Peroxide (e.g. hydrogen peroxide $H_2O_2$) is one of the key reactive oxygen species formed under oxidative stress conditions. High levels of peroxide formation have been linked to pathological conditions such as ageing, asthma, diabetes, atherosclerosis, cataract, inflammatory arthritis and neurodegenerative diseases.

Simple, direct and automation-ready procedures for quantitative determination of peroxide find wide applications in research and drug discovery. The Assay Genie ChromaDazzle Peroxide Assay kit is designed to measure peroxide concentration in biological samples without any pretreatment. The improved method utilizes the chromogenic $Fe^{3+}$-xylenol orange reaction, in which a purple complex is formed when $Fe^{2+}$ provided in the reagent is oxidized to $Fe^{3+}$ by peroxides present in the sample. The intensity of the color, measured at 540-610nm, is an accurate measure of the peroxide level in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

**KEY FEATURES**

**Sensitive and accurate**. Enhanced color intensity using sorbitol. Detection range 0.2 μM (7 ng/mL) to 30 μM (1,020 ng/mL) $H_2O_2$ in 96-well plate assay.

**Simple and high-throughput**. The procedure involves addition of a single detection reagent and incubation for 30 min. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

**APPLICATIONS:**

**Direct Assays:** $H_2O_2$ in biological samples (e.g. serum, citrate-plasma, urine, cell lysate, culture medium).

**Pharmacology:** effects of drugs on peroxide metabolism.

**KIT CONTENTS (250 tests in 96-well plates)**

**Reagent A: 1 mL**

**Reagent B: 50 mL**

**Standard: 100 μL** 3% stabilized $H_2O_2$.

Kit shipped at room temperature.

**Storage conditions**. The kit is shipped at room temperature. Store all reagents at 4 °C. Shelf life: 6 months after receipt.

**Precautions**: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

**PROCEDURES**

**Sample Treatment:** several chemicals are known to interfere and should be avoided in sample preparation. These include ascorbic acid, EDTA, heparin, DMSO (>0.02%), NP-40 (>0.6%), SDS (>0.12%), Tris (>8mM) and ethanol (>0.4%).

Samples can be analyzed immediately after collection, or stored in aliquots at –20 °C. Avoid repeated freeze-thaw cycles.

**Reagent Preparation:** Equilibrate to room temperature before assay. Prepare enough Detection Reagent by mixing 1 volume of Reagent A with 100 volumes Reagent B.

**Procedure using 96-well plate:**

1. *Standards*. Prepare fresh standards on the day of assay. Pipette 5 μL 3% $H_2O_2$ and mix well with 495 μL $H_2O$ in a 1.5-mL Eppendorf tube. Mix 5 μL of this solution with 1465 μL $H_2O$. The final $H_2O_2$ concentration is 30 μM (labeled “Premix”). Dilute standard as shown in the Table.

2. Transfer 40 µL diluted standards and each sample into separate wells of a clear flat-bottom 96-well plate. Add 200 µL Detection Reagent to all standards and samples.

| No | Premix + $H_2O$ | Vol (µL) | $H_2O_2$ (µM) |
|---|---|---|---|
| 1 | 100µL + 0µL | 100 | 30 |
| 2 | 80µL + 20µL | 100 | 24 |
| 3 | 60µL + 40µL | 100 | 18 |
| 4 | 40µL + 60µL | 100 | 12 |
| 5 | 30µL + 70µL | 100 | 9 |
| 6 | 20µL + 80µL | 100 | 6 |
| 7 | 10µL + 90µL | 100 | 3 |
| 8 | 0µL + 100µL | 100 | 0 |

3. Incubate 30 min at room temperature and read optical density at 540-610nm (peak absorbance at 585nm).

Note: if in rare cases, precipitation occurs after adding the Detection Reagent to a sample, transfer the whole reaction mixture of this sample well into a 1.5-mL Eppendorf tube and centrifuge 2 min at 14,000 rpm. Carefully remove 200 µL supernatant into a clean well and read OD. *Multiply the OD reading by 1.2 to account for the volume change*.

**CALCULATION**

Subtract blank OD (water, #8) from the standard OD values and plot the OD against $H_2O_2$ concentrations. Subtract blank OD from Sample OD. Determine the sample peroxide content from the standard curve.

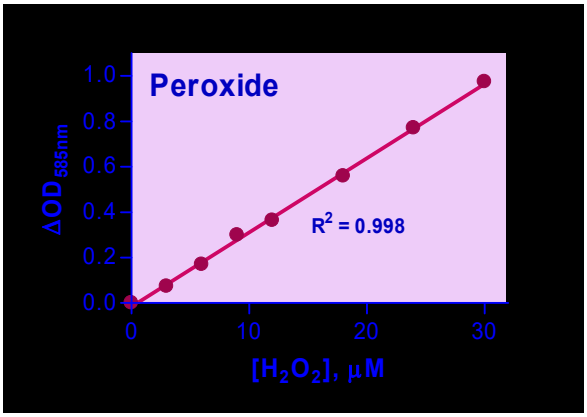
**Conversions**: 1 µM $H_2O_2$ equals 34 ng/mL or 34 ppb.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices and accessories, 96-well plates and plate reader.

**EXAMPLES:**

Duplicate assays for goat serum, human serum, 293 cell culture medium and fresh human urine gave peroxide content of 8.7± 2.8, 14.2 ± 2.7, 2.4 ± 0.0 and 1.4 ± 0.6 µM (n = 2).




**Standard Curve** in 96-well plate assay

**PUBLICATIONS**

[1]. Chi Li Yu et al (2008). A novel caffeine dehydrogenase in pseudomonas sp. strain CBB1 oxidizes caffeine to trimethyluric acid. J. Bacteriol. 190(2):772–776.

[2]. Deshmane, S.L. et al (2009). Activation of the oxidative stress pathway by HIV-1 Vpr leads to induction of hypoxia-inducible factor 1alpha expression. J Biol Chem. 284(17):11364-11373.

[3]. Giao, N.N. et al (2007). Water deficit induced pollen Sterility associated with a programmed cell death and oxidative stress in rice anthers. Proceedings the 2nd International Rice for the Future pp202-209.

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