



Hydrogen Peroxide Protocol

Version: 1
 Replaced by version
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Summary:

Hydrogen peroxide (H₂O₂) is a ubiquitous, toxic, metabolic by-product of aerobic respiration, oxidative stress, and oxidative injury. Cayman's Hydrogen Peroxide Assay Kit utilizes the well established xylenol orange detection method of quantifying the oxidation of ferrous ions (Fe²⁺) to ferric ions (Fe³⁺) by hydrogen peroxide. A unique feature of Cayman's assay is the inclusion of catalase as an H₂O₂ scavenger for the purpose of confirming the specificity of the reaction for H₂O₂. The sensitivity and the specificity of the assay make it well suited to accurately measure urinary levels H₂O₂ in a 96 well plate format. Each kit contains hydrogen peroxide, reagent 1, reagent 2, catalase, a 96 well plate, plate cover, and complete instructions.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	706011
Reagent 1&2		
Standard		
Catalase		

Protocol:

1. **H₂O₂ Standard Wells** - add 20 µl of standard (tubes A-G) and 10 µl of HPLC-grade water per well in the designated wells on the plate (see **Sample Plate Format**, Figure 1, page 7).
2. **Sample Wells** - Each sample should have at least two wells that will not contain catalase and two wells that will contain catalase. Add 20 µl of sample to the sample and sample + catalase wells. Then add 10 µl of catalase to the catalase wells and 10 µl of HPLC-grade water to the non-catalase wells.
3. Add 200 µl of Working Reagent to each well. Cover the plate with the plate cover and incubate on a shaker for one hour at room temperature.
4. Remove the plate cover and read the absorbance at 595 nm using a plate reader.

1. Calculate the average absorbance of each standard, sample, and sample + catalase.
2. Subtract the average absorbance of standard A from itself and from all other standards and samples including the catalase containing samples.
3. Plot the corrected absorbance of standards (from step 2 above) as a function of the final H₂O₂ concentration (µM) from Table 1. See Figure 2 (on page 13) for a typical standard curve.
4. Subtract the catalase sample absorbance from the non-catalase sample absorbance to yield the corrected sample absorbance.
5. Calculate the H₂O₂ concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample.

$$\text{H}_2\text{O}_2 \text{ (}\mu\text{M)} = \left[\frac{(\text{Corrected sample absorbance} - (\text{y-intercept}))}{\text{Slope}} \right] \times \text{Dilution}$$