Hydrogen Peroxide Protocol

Version: 1
Replaced by version
Edited by: Peter Havel - UC Davis Metabolism and Endocrinology Core

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. **H_2O_2 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 H_2O_2 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 H_2O_2 concentration of the samples using the equation obtained from the linear regression of the standard curve substituting corrected absorbance values for each sample.

$$H_2O_2\ (\mu M) = \left[\frac{(\text{Corrected sample absorbance} - (\text{y-intercept}))}{\text{Slope}} \right] \times \text{Dilution}$$