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UC Davis - Hydrogen Peroxide

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We use this protocol and it's working

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Abstract

Summary:

Hydrogen peroxide (H_2O_2) 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 H_2O_2 scavenger for the purpose of confirming the specificity of the reaction for H_2O_2. The sensitivity and the specificity of the assay make it well suited to accurately measure urinary levels H_2O_2 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.

Materials

MATERIALS

- Assay Kit **Cayman Chemical Company Catalog #**706011
- Reagent 1&2
- Standard
- Catalase

Note:

Cayman Chemical RRID:SCR_008945

Troubleshooting

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).

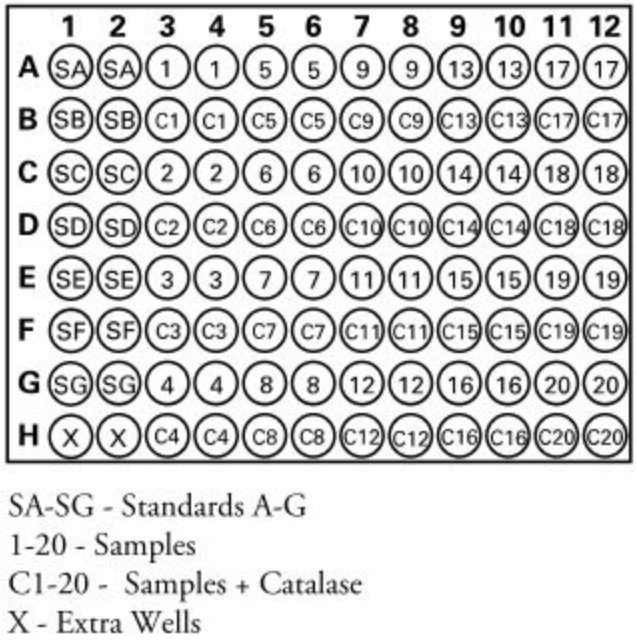

Figure 1. Sample plate format

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.

5 **Calculation**

1. Calculate the average absorbance of each standard, sample, and sample + catalase.

2. Substract 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.

Tube	Stock H_2O_2 (µl)	HPLC-grade water (µl)	Final Concentration (µM)
A	0	1,000	0
B	25	975	11
C	50	950	22
D	75	925	33
E	100	900	44
F	125	875	55
G	150	850	66

Table 1. H_2O_2 standards

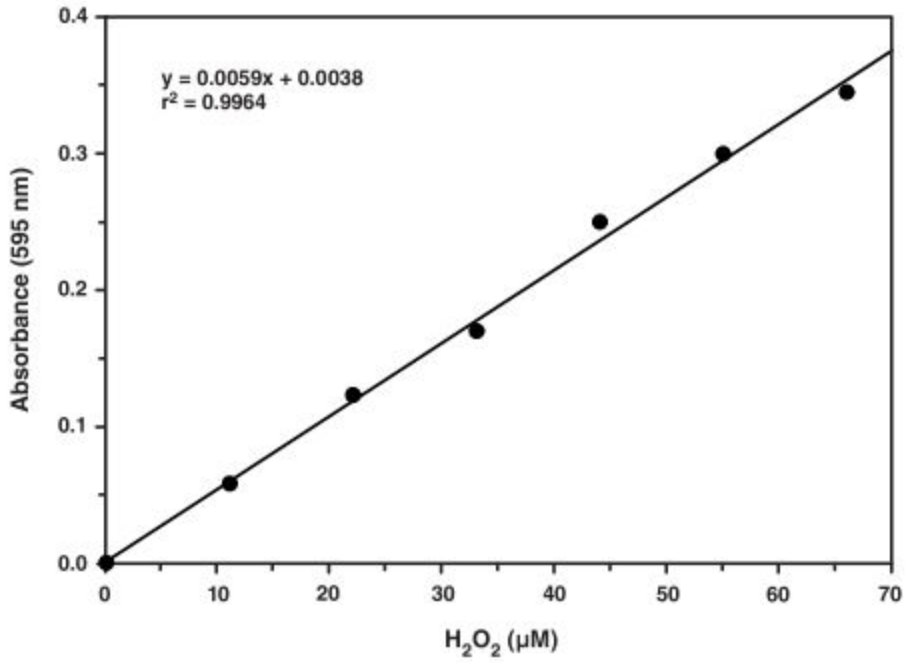

Figure 2. H_2O_2 Standard curve

4. Subtract the catalase sample absorbance from the non-catalase sample absprbance 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.

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