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 We use this protocol and it's working

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UC Davis - Hydrogen Peroxide

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ABSTRACT

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.

MATERIALS

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

Note:

Cayman Chemical [RRID:SCR_008945](#)

Keywords: Hydrogen
Peroxide

- 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).

	1	2	3	4	5	6	7	8	9	10	11	12
A	SA	SA	1	1	5	5	9	9	13	13	17	17
B	SB	SB	C1	C1	C5	C5	C9	C9	C13	C13	C17	C17
C	SC	SC	2	2	6	6	10	10	14	14	18	18
D	SD	SD	C2	C2	C6	C6	C10	C10	C14	C14	C18	C18
E	SE	SE	3	3	7	7	11	11	15	15	19	19
F	SF	SF	C3	C3	C7	C7	C11	C11	C15	C15	C19	C19
G	SG	SG	4	4	8	8	12	12	16	16	20	20
H	X	X	C4	C4	C8	C8	C12	C12	C16	C16	C20	C20

SA-SG - Standards A-G

1-20 - Samples

C1-20 - Samples + Catalase

X - Extra Wells

Figure 1. Sample plate format

- 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
- 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.
- 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. 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.

Tube	Stock H ₂ O 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. H₂O₂ standards

Figure 2. H₂O₂ 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.

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