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Product information

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Peroxide Quantitative Assay Kit (Lipid-Compatible)

Catalog #: BSP067
Size: 50 Test Tube Assays or 250 Microplate Assays
Storage: 4°C*

*: Product will be shipped at ambient temperature.
Upon receipt, store at 4°C.

Product Description:

The Peroxide Quantitative Assay Kit is a colorimetric assay that detects peroxide based on oxidation of ferrous to ferric ions in the presence of xylenol orange. The assays are suitable for H_2O_2 measurement in the following applications: quantitating detergent peroxide, monitoring cellular activity, and measuring peroxide accumulation in lipids. In the lipid compatible formulation, the peroxide converts Fe^{2+} to Fe^{3+} directly. In a sulfuric acid solution, the Fe^{3+} complexes with the xylenol orange dye to yield a purple product with maximum absorbency at 560 nm.

Contents:

Description	Size
Solution A	0.6ml
Solution B	60ml

Features:

- Quantitative range: 1-250µM
- Simple & Fast
- Colorimetric Assay

Reagent Preparation:

Working Reagent (WR):

Mix 1 volume Solution A with 100 volume of Solution B.

Notes: Prepare at least 1ml WR for each sample and standard replicate to be assayed in cuvettes. Prepare at least 200µl of WR for each sample and standard replicate to be assayed in a microplate.

The WR is stable for at least 12 hours.

Peroxide Standards:

Serially dilute a 30% (=8.8 M) hydrogen peroxide stock solution so as to achieve eight to ten standards in the concentration range 1-1000M.

Notes: A 30 % hydrogen peroxide solution can be standardized using 43.6 M-1cm-1 as the molar extinction coefficient for H_2O_2 at 240 nm. Then, prepare the high standard (1000µM) by adding 1µl of 30% hydrogen peroxide to 8.8 ml of water or buffer. The working range of the assay is from 1µM to 1mM peroxide in a sample assayed using the Standard Procedure (1:10 sample:WR ratio). Above 1mM, a bleaching effect occurs, causing a decrease in absorbance and loss of linearity in the assay. The standard curve is not linear over the entire assay range. Use a quadratic or best-fit curve to the standard points. Alternatively, use a linear fit for individual 100µM increments (e.g., from 1-100 µM, 101-200 µM, etc.); each incremental standard curve will be more horizontal in slope than the previous one until a plateau is reached at about 1mM.

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Standard Assay Procedure:

Note: If using this assay with lipid samples that are not extracted, prepare a blank that omits Solution A. Subtracting the absorbance for this blank from the sample tested in WR controls endogenous iron interferences. A blank may also be necessary if the sample contains other transition metals or a protein having chelating properties or strong absorbance characteristics at the wavelengths used for measurement. Excessive H_2O_2 (above 1mM) can result in low absorbance measurements caused by a bleaching effect on the dye. Ensure that results are accurate by preparing a 1:100 dilution of the sample and perform the assay in the same manner as the undiluted sample. If the absorbance of the diluted sample is higher than or similar to the original reading, then there is excessive peroxide in the sample. The Standard Assay Procedure uses a 1:10 sample:WR ratio. This ratio can be changed to accommodate samples with relatively high levels of peroxide (e.g., use 1:100 sample:WR) as long as the dilution is accounted for when comparing to the assay standards.

1. Add 10 volumes of WR to 1 volume of sample. For example, in a microplate, add 20µl sample (or standard) and 200 µl WR to each well.
2. Mix and incubate assay reactions for 15-20 minutes at room temperature. This incubation step is necessary for the reaction to reach an endpoint. Once formed, the complex is relatively stable, but it is best to measure the sample absorbances the same day as the reaction is performed.
3. Measure absorbance at 560-600nm (560 optimal) in a spectrophotometer, or at 595nm if using a plate reader.
4. Calculate the concentration of peroxide in the sample by reference to its assay absorbance compared to the standard curve (see notes in Reagent Preparation section for correct curve fitting procedures for the standards).

Procedure for Measuring Plasma or Serum Peroxide

Note: Plasma hydrogen peroxide levels may be determined using the lipid-compatible formulation in this kit (BSP067). Since plasma is quite low in peroxide content, the assay absorbance measurement often will be less than about 0.100. Because of endogenous plasma iron, a “TCEP Reference” must be assayed as described. This protocol is based on the work of Nourooz-Zadeh, J., *et.al*.4. Peroxide standards should be prepared as described in the Reagent Preparation section (use distilled water a blank).

1. For each plasma sample to be tested, pipette 90µl into each of two microcentrifuge tubes labeled “test” and “TCEP Reference.”
2. Add 10µl methanol to each “test” tube, and add 10µl of 10mM TCEP (BBI# TB0974) in methanol to each “TCEP Reference” tube.
3. Vortex tubes and incubate them for 20-30 minutes at room temperature.
4. To each tube, add 900µl WR.
5. Vortex tubes and incubate them for 20-30 minutes.
6. Centrifuge at about 12,000 x g for 5-10 minutes.
7. Transfer supernatant to either a cuvette (900µl) or a microwell plate (300µl) and measure the absorbances (see Standard Assay Procedure).
8. Calculate the peroxide content by comparing the difference between corresponding “test” and the “TCEP Reference” absorbances and the standard curve generated with hydrogen peroxide.

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NOT INTENDED FOR HUMAN OR ANIMAL USE.