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## Product information

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# Peroxide Quantitative Assay Kit (Water-Compatible)

**Catalog #:** BSP069
**Size:** 250 preps
**Storage:** 2~8°C*

*: Product will be shipped at ambient temperature. Check storage conditions.

## Product Description:

This Water-Compatible Quantitative Assay Kit uses a Colorimetric Assay to detect peroxide based on oxidation of ferrous to ferric ion in the presence of xylenol orange. Peroxide first reacts with sorbitol which enhances sensitivity by converting it to a peroxyl radical, this in turn initiates $Fe^{2}$+ oxidation to $Fe^{3}$+ at acidic pH, and the $Fe^{3}$+ complexes with the xylenol orange dye to yield a purple product with maximum absorbance at 560 nm. The complex displays maximal absorption between 540 and 580 nm, but also absorbs measurably up to 620 nm. When using microplates, the preferred wavelength for measurement is 595 nm (best signal-to-noise). The maximum absorbance of the assay components before addition of peroxide is at 430 nm. The kit is suitable for H2O2 measurement in many cellular assays, including quantitating detergent peroxide and monitoring cellular activity.

## Features:

- Quantitative range: 1-100 µM.
- Simple & fast.
- Colorimetric Assay. The kit is sufficient for 50 test tube assays or 250 microplate assays.

## Composition:

| | |
|---|---|
| Solution A | 0.6 ml |
| Solution B | 60 ml |

## Storage:

Product is shipped at room temperature. Upon receipt, store Solutions A and B at 2-8°C and protect from light.

## Reagent Preparation:

**1.** Working Reagent (WR): Mix 1 volume Solution A with 100 volumes Solution B (water-compatible formulation).

> **NOTE:** Prepare at least 1 ml WR for each sample and standard replicate to be assayed in cuvettes. Prepare at least 200 µl WR for each sample and standard replicate to be assayed in a microplate. The WR is stable for at least 12 hours.

**2.** Peroxide Standards: Prepare the high standard (1 mM) by adding 1 µl of 30% hydrogen peroxide to 8.8 ml of water or buffer. The working range of the assay is from 1 µM to 100 µM peroxide. Use this high standard to prepare standards within the working range of the assay. For example, add 1 µl, 10 µl, 20 µl, 40 µl, 60 µl, 80 µl, and 100 µl hydrogen peroxide (1 mM) into 8 separate 1.5 ml tubes, then add $DDH_2O$ until total volume is 1000 µl.

 

## Standard Assay Procedure

**1.** Add 10 volumes of WR to 1 volume of sample. For example, in a microplate, add 20 µl sample (or standard) and 200 µl WR to each well. For test tubes add 100 µl sample (or standard) and 1000 µl WR to each tube).

**2.** Mix and incubate assay reactions for 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 the reaction is performed.

**3.** Measure absorbance at 560 nm in a spectrophotometer or at 595 nm if using a plate reader.

**4.** Calculate the concentration of peroxide in the sample by comparing its assay absorbance with the standard curve .

## Additional Notes:

**1.** If using this assay with samples that contain transition metals (e.g. iron), prepare a blank that omits Reagent A. Subtracting the absorbance for this blank from the sample tested in WR controls for endogenous iron interference. A blank may also be necessary if the sample contains other transition metals or if it is a protein with chelating properties or strong absorbance characteristics at the wavelengths used for measurement.

**2.** Excessive H2O2 (above 1 mM) 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.

**3.** The Standard Assay Procedure uses a 1:10 sample:WR ratio. However, 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.