Version 1b Last updated 6 August 2025

ab272537
Peroxide Assay Kit

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Peroxide Assay Kit datasheet:
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For quantitative determination of peroxide concentration in biological samples without any pretreatment.

This product is for research use only and is not intended for diagnostic use.

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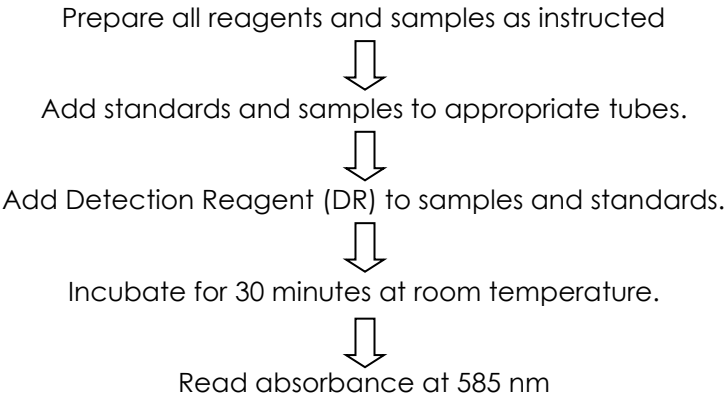
1. Overview

Peroxide Assay Kit (ab272537) is a simple, direct and automation-ready procedures for quantitative determination of peroxide find wide applications in research and drug discovery. This assay kit is designed to measure peroxide concentration in biological samples without any pretreatment. The improved method utilizes the chromogenic Fe^{3+}-xylenol orange reaction, in which a purple complex is formed when Fe^{2+} provided in the reagent is oxidized to Fe^{3+} by peroxides present in the sample. The intensity of the color, measured at 540-610nm, is an accurate measure of the peroxide level in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

Sensitive and accurate: Enhanced color intensity using sorbitol. Detection range 0.2 µM (7 ng/mL) to 30 µM (1,020 ng/mL) H_2O_2 in 96-well plate assay.

Simple and high-throughput: The procedure involves addition of a single detection reagent and incubation for 30 min. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent A	1 mL	+4°C
Reagent B	50 mL	+4°C
Standard (3% stabilized H_2O_2)	100 µL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H_2O
- Multi-channel pipette
- 1.5 mL tubes
- 1.5 mL centrifuge
- 96-well clear plate with flat bottom
- Standard microplate reader - capable of reading absorbance at 540-610 nm (peak absorbance is at 585 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 250 assays.

All reagent are supplied ready to use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

Premix Standard:

10.1.1 In a 1.5 mL Eppendorf tube, mix 5 µL Standard (3% H_2O_2) and 495 µL H_2O to obtain a 1:100 dilution.

10.1.2 Prepare 1470 µL 30 µM Premix by mixing 5 µL H_2O_2 (1:100 dilution) and 1465 µL distilled water.

10.1.3 Dilute standards in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Premix (µL)	H_2O (µL)	H_2O_2 (µM)
1	100	0	30
2	80	20	24
3	60	40	18
4	40	60	12
5	30	70	9
6	20	80	6
7	10	90	3
8	0	100	0

11. Sample Preparation

Sample treatment:

Serum, citrate-plasma, urine and culture media can be assayed directly.

11.1 Cell cultures:

11.1.1 Collect cells by centrifugation 1,000 g for 10 min at 4°C. For adherent cells, use a rubber policeman to harvest cells.
11.1.2 Homogenize or sonicate cell pellet in 1-2 mL of cold buffer containing 0.9% sodium chloride and 5 mM potassium phosphate, pH 7.4.
11.1.3 Centrifuge at 10,000 x g for 10 min at 4°C.
11.1.4 Use the clear supernatant for assay. If not assaying on the same day, freeze the sample at -80°C and use it within one month.

Δ Note: Stored samples in aliquots at –20 °C. Avoid repeated freeze-thaw cycles.

Δ Note: Several chemicals are known to interfere and should be avoided in sample preparation. These include ascorbic acid, EDTA, heparin, sodium pyruvate (>1mM), DMSO (>0.02%), NP-40 (>0.6%), SDS (>0.12%), Tris (>8mM), and ethanol (>0.4%).

Δ Note: If the sample contains chemicals known to interfere with the assay, if the expected concentration of H_2O_2 is sufficiently high, the sample can be diluted to reduce the level of the chemicals to a concentration below that at which they will interfere with the assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Detection Reagent:

12.1.1 Prepare enough Detection Reagent (DR) for all samples and standards by mixing per reaction well: 2 µL Reagent A and 200 µL Reagent B.

Component	Working Reagent (µL/reaction)
Reagent A	**2**
Reagent B	**200**

12.1.2 Add 40 µL of Standards and Samples to separate wells.
12.1.3 Add 200 µL of Detection Reagent to each Sample and Standard well.
12.1.4 Incubate for 30 min at room temperature.
12.1.5 Read OD at 540-610 nm (peak 585 nm).

Δ Note: If in rare cases, precipitation occurs after adding the Detection Reagent to a sample, transfer the whole reaction mixture of this sample well into a 1.5-mL Eppendorf tube and centrifuge 2 min at 14,000 rpm. Carefully remove 200 µL supernatant into a clean well and read OD. Multiply the OD reading by 1.2 to account for the volume change

13. Calculations

13.1.1 Subtract Standard #8 (H_2O) OD value from the remaining Standard OD values and plot against H_2O_2 concentrations.

13.1.2 Determine the sample peroxide content from the standard curve.

Conversions: 1 µM H_2O_2 equals 34 ng/mL or 34 ppb.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

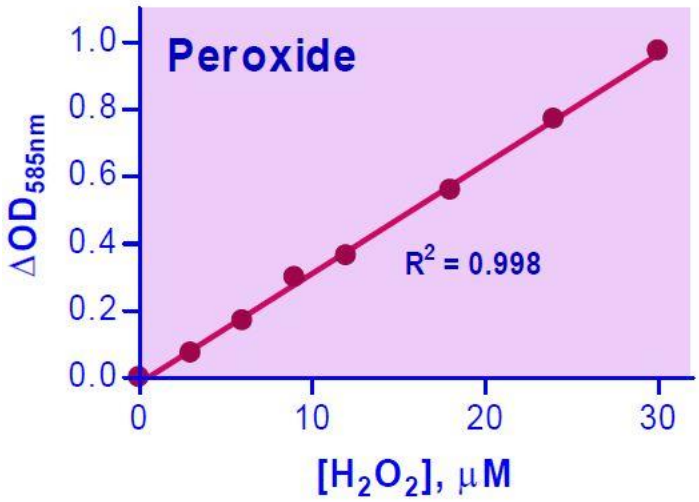


Figure 1. Example of Peroxide Assay Kit standard curve.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/en-us/contact-us

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)