ab155895

Peroxidase Activity Assay Kit

Instructions for Use

For the sensitive and accurate measurement of peroxide activity in biological samples.

View kit datasheet: www.abcam.com/ab155895
(use www.abcam.cn/ab155895 for China, or www.abcam.co.jp/ab155895 for Japan)

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

Version 6b, Last Updated 8 January 2026

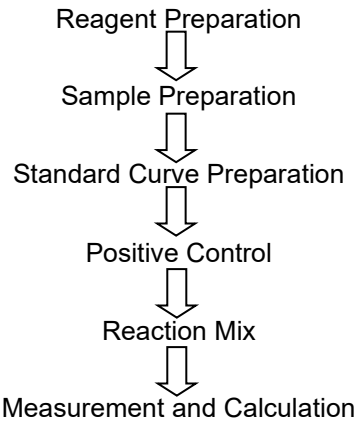
Table of Contents

1. Overview

Peroxidases (EC number 1.11.1.x) are a large family of enzymes that typically catalyze a reaction of the form: ROOR' + electron donor (2 e-) + 2H+ → ROH + R'OH. For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such as lipid peroxides. Peroxidases can contain a heme cofactor in their active sites, or alternately redox-active cysteine or selenocysteine residues.

Abcam's Peroxidase Assay Kit provides a convenient colorimetric and fluorometric means to measure the peroxidase activity in biological samples. In the presence of Peroxidase, the OxiRed Probe reacts with H_2O_2 in a 1:1 stoichiometry to produce the redfluorescent oxidation product, resorufin. The resorufin is quantified by colorimetric (λmax = 570nm) or fluorometric methods (Ex/Em = 535/587 nm). The assay is simple, direct, highly sensitive and high throughput-ready. The detection limit is 0.1 mU per well via colorimetric or 0.01 mU per well via fluorometric method, based on our unit definition.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 18	25 mL
OxiRed™ Probe	0.2 mL
H2O2 Standard	100 µL
Developer Solution V	1 vial

PLEASE NOTE: Assay Buffer 18 was previously labelled as Assay Buffer XVIII and Assay Buffer, and OxiRed™ Probe as OxiRed Probe and OxiRed™ Probe (in DMSO). The composition has not changed.

* Store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm Assay Buffer 18 to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

- 96-well clear plate with flat bottoms
- Multi-well spectrophotometer (ELISA reader)

4. Assay Protocol

A. Reagent Preparation

1. H2O2 Standard:

Dilute H2O2 Standard to 12.5 mM by adding 5 µl of H2O2 Standard (0.88 M) to 347 µl Assay Buffer 18 The diluted H2O2 Standard is stable for one day at 4°C and one month at -20°C.

2. Developer Solution V:

Add 1 ml Assay Buffer 18 into lyophilized Developer Solution V to prepare diluted Developer Solution V. The HRP solution is stable for one day at 4°C and one month at -20°C.

3. OxiRed™ Probe:

Before use, briefly warm at 37°C for 1-2 min to completely melt DMSO solution, mix well. Store at –20°C.

B. Peroxide Assay Protocol

1. Sample Preparation:

Collect cell culture supernatant, serum, plasma, urine, and other biological fluids. Centrifuge test samples for 15 minutes at 1000 x g within 30 min of collection to remove particulate pellet. Assay immediately or aliquot and store the samples at -80°C. Avoid repeated freeze-thaw cycles. Add 2-50 µl samples into each well and adjust the final volume to 50 µl with Assay Buffer 18.

2. **Standard Curve Preparations:**

For colorimetric assay:

Dilute H2O2 Standard solution to 0.1 mM by adding 10 µl of H2O2 Standard solution (12.5 mM) to 1240 µl Assay Buffer 18, mix well. Add 0, 10, 20, 30, 40, 50 µl into a series of wells in duplicate and adjust the final volume to 50 µl with Assay Buffer 18 to generate 0, 1, 2, 3, 4, 5 nmol/well of H2O2 Standard.

For fluorometric assay:

Dilute H2O2 Standard solution to 0.01 mM by adding 100 µl of H2O2 Standard solution (0.1 mM) to 900 µl Assay Buffer 18, mix well. Add 0, 10, 20, 30, 40, 50 µl into a series of wells in duplicate and adjust the final volume to 50 µl with Assay Buffer 18 to generate 0, 100, 200, 300, 400, 500 pmol/well of H_2O_2 standard.

3. **Standard Curve Measurement:**

Dilute Developer Solution V 1:199 in Assay Buffer 18. For each well, prepare a total 50 µl Reaction Mix containing 2 µl OxiRed™ Probe and 48 µl diluted Developer Solution V, mix well. Incubate

for 5 min and measure the OD at 570 nm or RFU at Ex/Em = 535/587 nm in a micro plate reader.

4. Positive Control Preparation:

Use 1 µl of diluted Developer Solution V into the desired well(s) and adjust the final volume to 50 µl with Assay Buffer 18.

5. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

Assay Buffer 18	46 µl
OxiRed™ Probe solution	2 µl
H2O2 Standard solution	2 µl

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading/increase detection sensitivity significantly.

Add 50 µl of the Reaction Mix to each test samples and Developer Solution V. Mix well; incubate the mix for 3 min at 37°C.

6. Measurement

Measure OD 570 nm (A0) for colorimetric assay or Ex/Em = 535/587 nm (R0) for fluorometric assay. Incubate for another 30 min to 2 hr at 37°C to measure OD at 570 nm (A1) or fluorescence

at Ex/Em = 535/587 nm (R1) again, incubation times will depend on the peroxidase activity in the samples. We recommend measuring the OD or fluorescence in a kinetic method (preferably every 3 – 5 min) and choose the period of linear range, which falls within H_2O_2 Standard Curve to calculate the peroxidase activity of the samples.

5. Data Analysis

Calculation: Plot the H_2O_2 Standard Curve. Calculate the Peroxidase activity of the test samples: $\Delta A = A_1 - A_0$ or $\Delta RFU = R_1 - R_0$, apply the ΔA or ΔRFU to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated by peroxidase in the given time.

$$\textbf{Peroxidase Activity} = \frac{\textbf{B}}{\textbf{(T x V)}} \times \textbf{Sample Dilution Factor} = \textbf{nmol/min/ml = mU/ml}$$

Where:

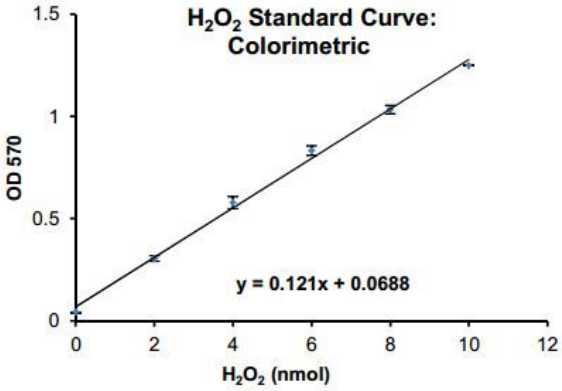
B is the amount of H_2O_2 from standard curve (nmol).

ΔT is the time incubated (min).

V is the sample volume added into the reaction well (ml).

Unit Definition: One unit of Peroxidase is the amount of enzyme that will oxidize 1.0 µmol of H_2O_2 per min at 37°C.

(a)



(b)

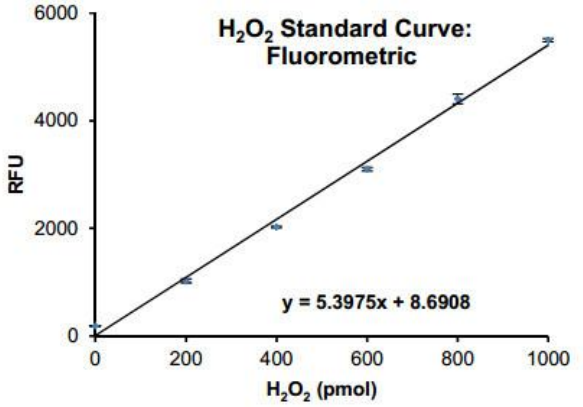


Figure 1: (a) H_2O_2 standard curve - colorimetric (b) H_2O_2 standard curve - fluorometric. Assays were performed following kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)

Problem	Reason	Solution
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix

Problem	Reason	Solution
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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