ab324119 – Peroxidase Assay Kit (Luminometric)

Can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screening of oxidase inhibitors, etc.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324119

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	1 bottle (25 mL)	-20°C
H_2O_2	1 vial (3% stabilized solution, 200 µL)	+4°C (Store in the dark)
Horseradish Peroxidase	1 vial (20 units)	-20°C (Store in the dark)

Materials Required, Not Supplied

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

- Luminescence microplate reader
- Solid black/ white plates
- PBS with 0.1% BSA

Protocol Summary

1. Prepare HRP working solution (50 µL).
2. Add HRP standards and/or test samples (50 µL).
3. Incubate at room temperature for 30 minutes to 2 hours.
4. Monitor luminescent intensity.

IMPORTANT: Thaw all kit components at room temperature before starting the experiment.

Preparation of Stock Solutions

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

HRP stock solution (20 U/mL)

1. Add 1 mL of PBS with 0.1% BSA into the vial of Horseradish Peroxidase.

Preparation of Standard Solution

HRP standard

Add 1 µL of 20 U/mL HRP stock solution in 1999 µL of PBS with 0.1% BSA to get 10 mU/mL HRP standard solution (PS7). Then use 10 mU/mL standard solution and perform 1:2 serial dilutions to obtain remaining serially diluted standards (PS6-PS1).

Preparation of Working Solution

1. Add 30 µL of 3% stabilized H_2O_2 solution into 5 mL of Assay Buffer to make HRP working solution and keep from light.

***Note**: The HRP working solution is stable at room temperature for at least 8 hours without activity loss if kept from light.*

Experimental Protocol

BL	BL	TS	TS
PS1	PS1	…	…
PS2	PS2	…	…
PS3	PS3		
PS4	PS4		
PS5	PS5		
PS6	PS6		
PS7	PS7		

Table 1. Layout of HRP standards and test samples in a solid black 96-well microplate. PS=Peroxidase Standards (PS1-PS7, 0.156 to 10 mU/mL); BL = blank control; TS = test sample.

Well	Volume	Reagent
PS1-PS7	50 µL	Serial Dilution (0.156 to 100 mU/mL)
BL	50 µL	PBS with 0.1% BSA
TS	50 µL	Test Sample

Table 2. Reagent composition for each well of a 96-well microplate.

1. Prepare HRP standards (PS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of HRP working solution to each well of HRP standard, blank control, and test samples to make the total HRP assay volume of 100 µL/well. For a 384-well plate, add 25 µL of HRP working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction at room temperature for 30 minutes to 2 hours, protected from light.
4. Monitor the luminescence intensity by using a standard luminometer.

Data Analysis

The reading (RLU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation.

For technical support contact information, visit: www.abcam.com/contactus

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