CREATIVE BIOMART®
Assay Kit

Fluorimetric Peroxidase Assay Kit (Near Infrared Fluorescence)

Product Information

Cat

Kit-0993

Common Name

Peroxidase

Cat.No.

Kit-0993

Product Overview

We offer this quick HRP assay in a one-step, homogeneous, no wash assay system. Our Fluorimetric Peroxidase Assay Kit uses IR, our near infrared flurogenic HRP substrate to quantify peroxidase in solution. IR generates a substance that has maximum absorption of 647 nm with maximum emission at 670 nm. This near infrared absorption and fluorescence minimize the assay background that is often caused by the autoabsorption and/or autofluorescence of biological samples that rarely absorb light beyond 600 nm. The kit can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screenings, etc. The kit provides an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. It can detect as low as 1 mU/mL HRP. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 600 to 650/650 to 690 nm (maximum Ex/Em = 640 /680 nm) or an absorbance microplate reader at 647±5 nm.

Description

Horseradish Peroxidase (HRP) is a small molecule (MW ~40 KD) that is widely used in a variety of biological detections. HRP conjugates are extensively used as secondary detection reagents in ELISAs, immunohistochemical techniques, Northern, Southern and Western blot analyses. Due to its small size, it rarely causes steric hindrance problem with the antibody/antigen complex formation. It is usually conjugated to an antibody in a 4:1 ratio. Meanwhile, HRP is inexpensive compared to other labeling enzymes.

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Storage

Keep in freezer and avoid exposure to light.

Size

500 assays

Kit Components

Component A: IR Peroxidase Substrate 1 vial
Component B: H2O2 1 vial (3% stabilized solution, 200 μL)
Component C: Assay Buffer 1 bottle (100 mL)
Component D: Horseradish Peroxidase 1 vial (20 units)
Component E: DMSO 1 vial (1 mL)

Features & Benefits

Broad Application: Can be used for quantifying HRP activities in solutions and solid surfaces (e.g, ELISA)
Sensitive: Detect as low as 1 mU/mL HRP in solution.
Continuous: Easily adapted to automation without a separation step.
Convenient: Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive: No special requirements for waste treatment.

Preparation

1. Prepare stock solutions:
1.1 100X IR peroxidase substrate stock solution: Add 250 uL of DMSO (Component E) into the vial of IR Substrate (Component A). The stock solution should be used promptly, and any remaining solution should be aliquoted and refrozen at -20°C.
Note: Avoid repeated freeze-thaw cycles.
1.2 20 U/mL HRP stock solution: Add 1 mL of Assay Buffer (Component C) into the vial of HRP (Component D).
Note: The unused HRP solution should be divided into single use aliquots and stored at -20°C.
1.3 20 mM H2O2 stock solution: Add 22.7 uL of 3% H2O2 (0.88 M, Component B) into 977 uL of Assay Buffer (Component C).

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Note: The diluted H2O2 solution is not stable. The unused portion should be discarded.
2. Prepare HRP reaction mixture: Prepare HRP reaction mixture according to the following table and keep from light.
Table 1. HRP reaction mixture for one 96-well plate (2X)
IR peroxidase substrate stock solution (100X, from Step 1.1) 50 uL
20 mM H2O2 stock solution (from Step 1.3) 50 uL
Assay buffer (Component C) 4.9 mL
Total volume 5 mL
3. Prepare serially diluted HRP standards (0 to 300 mU/mL):
Warnings: 1. The component A is unstable in the presence of thiols such as DTT and β-mercaptoethanol. The presence of thiols at concentration higher than 10 μM would significantly decrease the assay dynamic range.
2. NADH and glutathione (reduced form: GSH) may interfere with the assay.
3.1 Add 15 μL of 20 U/mL HRP stock solution (from Step 1.2) into 985 μL of Assay Buffer (Component C) to get 300 mU/mL HRP standard solution.
3.2 Take 200 μL of 300 mU/mL HRP standard solution to perform 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3 and 0 mU/mL serially diluted HRP standards.
3.3 Add serially diluted HRP standards and/or peroxidase-containing test samples into a solid black 96-well microplate as described in Tables 2 and 3.
Table 2. Layout of HRP standards and test samples in a solid black 96-well microplate
BL BL TS TS …. ….
PS1 PS1 …. …. …. ….
PS2 PS2
PS3 PS3
PS4 PS4
PS5 PS5
PS6 PS6
PS7 PS7
Note: PS=Peroxidase Standards; BL=Blank Control; TS=Test Samples
Table 3. Reagent composition for each well:

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HRP Standards: Serial Dilutions*: 50 μL
Blank Control: Assay Buffer (Component C): 50 μL
Test Sample: 50 μL
*Note: Add the serially diluted peroxidase standards from 0.3 mU/mL to 300 mU/mL into wells from PS1 to PS7 in duplicate.

Assay Protocol

4. Run HRP assay in supernatants:
4.1 Add 50 μL of HRP reaction mixture (from Step 2) into each well of HRP standard, blank control, and test samples (see Step 3.3) to make the total HRP assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 μL of sample and 25 μL of HRP reaction mixture into each well.
4.2 Incubate the reaction at room temperature for 30 to 60 minutes, protected from light.
4.3 Monitor the fluorescence increase with a fluorescence plate reader at excitation 600-650 nm (optimal at 640) with emission at 650-690 nm (optimal at 680).
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 647±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the peroxidase reactions.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

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