Colorimetric Peroxidase Assay Kit (Red)

Cat. No. Kit-0992 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	We offer this quick (10 min) HRP assay in a one-step, homogeneous, no wash assay system. This kit uses our fluorogenic Red HRP substrate to quantify peroxidase in solutions. It can be used for ELISAs, characterizing kinetics of enzyme reaction and high throughput screenings, etc. This Fluorimetric Peroxidase Assay Kit provides an optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments. It can detect as low as 10 uU/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 = 540±10/590 ±10 nm (maximum Ex/Em = 540/590 nm) or an absorbance microplate reader at 576±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. Additionally, HRP is inexpensive compared to other labeling enzymes. The major disadvantage associated with peroxidase is their low tolerance to many preservatives such as sodium azide that inactivates peroxidase activity even at low concentration.
Storage	Keep in freezer and avoid exposure to light.
Size	500 assays

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Kit Components	Component A: Red 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 10 uU/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 Red peroxidase substrate stock solution: Add 250 uL of DMSO (Component E) into the vial of Red 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). 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) Red peroxidase substrate stock solution (100X, from Step 1.1) 50 uL

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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 10 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 1 μL of 20 U/mL HRP stock solution (from Step 1.2) into 1999 μL of Assay Buffer (Component C) to get 10 mU/mL HRP standard solution.
3.2 Take 200 μL of 10 mU/mL HRP standard solution to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.01 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:
HRP Standards: Serial Dilutions*: 50 μL
Blank Control: Assay Buffer (Component C): 50 μL
Test Sample: 50 μL

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	Note 1: Add the serially diluted HRP standards from 0.01 mU/mL to10 mU/mL into wells from PS1 to PS7 in duplicate. Note 2: High levels of HRP (e.g., >100 mU/mL final concentration) may cause reduced fluorescence signal due to the over oxidation of Red (to non-fluorescent one).
Assay Protocol	4. Run HRP assay in supernatants: 4.1 Add 50 µL of HRP reaction mixture (from Step 2) to each well of HRP standard, blank control, and test samples (see Step 3.3) to make the total HRP assay volume of 100 µ/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 mixture at room temperature for 15 to 30 minutes, protected from light. 4.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540±10/590± 10 nm (optimal Ex/Em = 540/590). 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 576±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 subtracted from the values for those wells with the HRP 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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