Peroxidase Activity Assay

Catalog Number	Kit Size
TBS2067-100	100 assays
TBS2067-200	200 assays

DESCRIPTION

10-Acetyl-3,7-dihydroxyphenoxazine (ADHP), also called Amplex Red or Ampliflu Red, is a sensitive and stable fluorogenic substrate for horseradish peroxidase (HRP). In the presence of HRP and H_2O_2, ADHP generates highly fluorescent resorufin in red color that has a maximum absorption of 570 nm and maximum emission of 585 nm. So far ADHP has been known as the most sensitive and stable fluorogenic probe for detecting HRP and H_2O_2. It has been widely used to detect peroxidase Activity.

TribioScience's Peroxidase Activity Fluorometric / Colorimetric Assay provides a quick, efficient, and sensitive method of peroxidase activity.

APPLICATION

Detection of HRP activity on liquid system

FEATURES

- Sensitive and accurate: 10 times more sensitive than TMB substrate for HRP detection.
- Simple: Just add- incubate-read style.

KIT CONTENTS

Compent	100 Assays	200 Assays
ADHP solution	60 µL	120 µL
Developer	600 µL	1200 µL
HRP standard (1 U/mL)	50 µL	100 µL
1X Assay buffer	10 ml	20 mL

Storage Conditions: Store the kit at –20°C, protected from light.
Shelf Life: 12 months.

ASSAY PROCEDURES

Solution Preparation

1. Standard Curve Preparations (Fig. 1):

1.1 Label 1.5mL Std tubes 1-8. As shown below in the diagram.

1.2 Add 495 µL of 1x Assay Buffer to Std1 and 200µL to Std 2-8.

1.3 Add 5 µL of HRP Stock Solution (1U/mL) to Std1, and then make 2x serial dilution from Std#2 to 7 by transferring 200 µL of the higher concentration to the next one. Leave Std#8 in 1x Assay Buffer as 0 standard. The standard concentration range is 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625mU/mL, and 0.

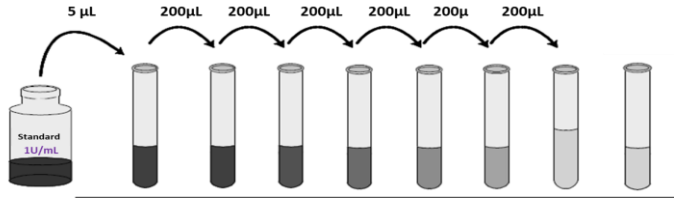
2. ADHP/Developer Working Solution Preparation:
   - 50 µL of ADHP stock solution
   - 500 µL of Developer.
   - 4.45 mL of 1X Reaction Buffer

   This 5 mL volume is sufficient for ~100 assays.

Assay Procedures

3. Pipet 50 µL of standard, samples, controls, into individual wells of a microplate.

Fig.1: Diagram for HRP Standard Preparation



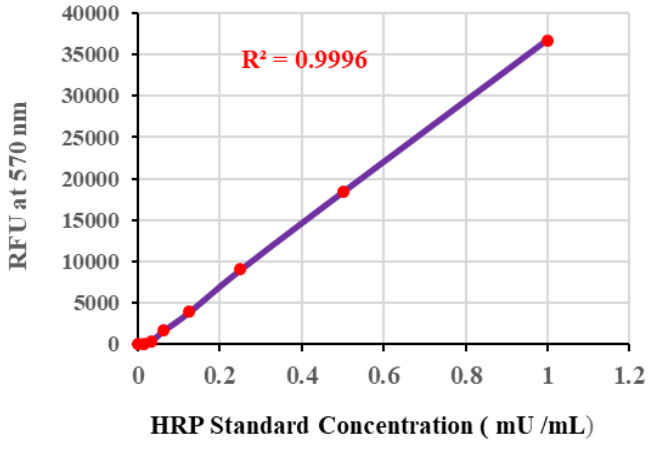
	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	495	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	5	200	200	200	200	200	200	
Final Conc (mU/mL)	10	5	2.5	1.25	0.625	0.3125	0.15625	0

4. Add 50 µL of ADHP/Developer working solution (prepared in Step 2) to each well.
5. Incubate at RT for 30 min.
6. Measure the fluorescence or absorbance: Use a microplate reader equipped for excitation in the range of 530-560 nm and fluorescence emission detection at ~590 nm, or for absorbance at ~570 nm.
7. Correct for background fluorescence or absorbance. For each point, subtract the value derived from the no-HRP control.

Calculation

Subtract the blank value (0 mU/mL Standard) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate HRP concentration of the Sample using the equation obtained from the linear regression of the standard curve. HRP (mU/mL) = N x (Rsample- Rblank)/Slope. Where: Rsample and Rblank are optical density or fluorescence intensity readings of the sample and blank, respectively. N is the sample dilution factor.

Fig.2 HRP Fluorometric Assay



RELATED PRODUCTS:

ATP Activity Assay (TBS2010)
Amplex Red Hydrogen Peroxide Assay Kit (TBS2066)
AmplexRed_HRP-System（TBS5026）
Tryptase Activity Assay (TBS2101)
β-Hexosaminidase Activity Assay (TBS2105)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

For research use only.

Fig.3: HRP Colorimetric Assay