Fast Hydrogen Peroxide Assay

Fluorometric and Colorimetric Assay

Catalog Number	Kit Size
TBS2066-100	100 assays
TBS2066-200	200 assays

DESCRIPTION

Hydrogen Peroxide (H_2O_2) is a reactive oxygen metabolic byproduct that serves as a key regulator for a number of oxidative stress-related states. It is involved in many pathological processes of diseases such as asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, neuro-degenerative diseases, Down's syndrome, and immune system diseases.

Tribioscience's ADHP-red Hydrogen Peroxide Assay Kit is a sensitive, simple, direct, and HTS-ready colorimetric/fluorometric assay designed to measure H_2O_2 levels in biological samples. In the presence of Horse Radish Peroxidase (HRP), the ADHP-red probe reacts with H_2O_2 to produce a resorufin that appears red in color. The resorufin can be measured with absorption of 570nm (λ_{max} = 570nm) for colorimetric or a highly stable fluorometric assay at maximum emission of 585nm (Ex/Em = 535/587 nm).

APPLICATION

- Detection of HRP activity on liquid systems such as ELISA.
- Western Blot or other immunoassays.
- Custom packaging and bulk purchase information are available upon request

FEATURES

- Flexible: Suitable for colorimetric and fluorometric methods.
- Accurate: Use 50 µL samples. Detection ranges 0.4-200 µM in 96-well plate for colorimetric assay and fluorometric assay.
- Simple and high-throughput: One-step procedure: just load-incubate-Read. The kit can be used for a robust method.
- Time-saving less than 30 minutes

KIT CONTENTS

Component	100x Rxns	200x Rxns
ADHP solution	60µL	120µL
H_2O_2 Standard (0.88M)	50µL	100µL
HRP Reagent	100µL	200µL
1x Reaction buffer	10ml	20mL

Storage conditions: Store the kit at –20°C protected from light.
Shelf life: 6 months.

ASSAY PROCEDURES

1. Sample Preparation

Collect cell culture supernatant, serum, plasma, urine and other biological fluids. Centrifuge for 5-minutes at 5000 xg then collect supernatant for analysis. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C. Avoid repeated freeze-thaw cycles. Add 50µl samples into each well, then bring the volume to 100µl with assay buffer.

2. Standard Preparation:

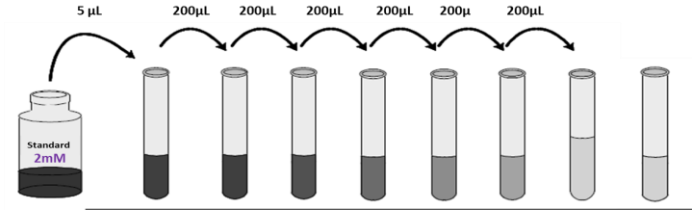
2.1 Dilute 2.0µL of 0.88M H_2O_2 into 878µL of 1x Assay buffer to generate a 2mM H_2O_2 stock solution (*Note: 2 mM H_2O_2 stock solution prepared in this step will be less stable and should be used within a few hours of preparation, although the H_2O_2 stock solution (0.88M) has been stabilized to slow its degradation*).

2.2 Label 8x 1.5mL tubes 1-8 for a standard curve as shown in the diagram below.

Add 995µL of 1x Assay Buffer to Std 1 and 200µL to Std 2-8.

2.3 Add 5µL of 2mM H_2O_2 Stock solution to Std1 then transfer 200µL of Std1 to Std2. Carry out a 2x serial dilution for Std 3-7. Leave Std 8 as the 0 standard (the assay buffer alone). The standards concentrations are 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, and 0 µM for Std 1-8.

Fig.1 Diagram for H2O2 Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	995	200	200	200	200	200	200	200
Addition	2mM Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol.(µL)	5	200	200	200	200	200	200	
Final Conc (µM)	10	5	2.5	1.25	0.625	0.3125	0.15625	0

2.4 Load the samples: Pipet 50µL of standards, controls, and test samples into individual wells of a microplate in duplicate manner *(Note: recommend to run a pilot study to determine the optimal concentration of sample within the assay standard curve range)*.

2.5 Prepare ADHP-red working solution by mixing the reagent as following:

• 50µL ADHP-red reagent stock solution
• 100µL HRP stock solution
• 4.85mL of 1x Reaction Buffer

This 5mL volume is sufficient for ~100 assays. Note that the final concentration of each component will be two-fold lower in the final reaction volume.

2.6 Begin the reactions. Add 50µL of above ADHP-red working solution to each microplate well containing the standards, controls, and samples.

2.7 Incubate the reactions. Incubate at room temperature for 30-minutes protected from light. Because the assay is continuous (not terminated), you may measure fluorescence or absorbance at multiple time points to follow the kinetics of the reactions.

2.8 Measure fluorescence or absorbance using a microplate reader with excitation range of 530-560 nm and fluorescence

emission detection at ~590nm, or absorbance at ~560nm.
2.9 Correct for background fluorescence or absorbance. For each point, subtract the value derived from the no-H_2O_2 control.

Calculation

Subtract the blank value (0μM Standard) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the glucose concentration of the Sample using the equation obtained from the linear regression of the standard curve. H_2O_2 = N x (Rsample- Rblank)/Slope (μM)
Where: Rsample and Rblank are optical density or fluorescence intensity readings of the sample and blank, respectively. N is the sample dilution factor.

The Typical data is displayed in Fig.2 and Fig 3.

RELATED PRODUCTS:

ATP Activity Assay (TBS2010)
ADHP Red Hydrogen Peroxidase Assay Kit (TBS2067)
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)

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