USER GUIDE

For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

Mes-Red™过氧化氢/过氧化物酶检测试剂盒
Mes-Red™ Hydrogen Peroxide/Peroxidase Assay Kit

Do not eat Store at -20° C & in the dark.

Cat.No. MHP4684 **Size :** 500 tests

Technical literature is available at : www.mesgenbio.com.
E-mail MesGen Technical Services if you have questions on use of this system : tech@mesgenbio.com

Description

Mes-Red Hydrogen Peroxide/Peroxidase Assay Kit could use the Mes-Red reagent to detect hydrogen peroxide (H_2O_2) or peroxidase activity. The Mes-Red reagent, in combination with horseradish peroxidase (HRP), has been used to detect H_2O_2 released from biological samples, including cells, or generated in enzyme-coupled reactions. Furthermore, Mes-Red reagent can be used as an ultrasensitive assay for peroxidase activity when H_2O_2 is in excess. In the presence of peroxidase, the Mes-Red reagent reacts with H_2O_2 in a 1:1 stoichiometry to produce the red-fluorescent oxidation product, resorufin. Resorufin has excitation and emission maxima of approximately 570 nm and 585 nm, respectively, and because the extinction coefficient is high, you can perform the assay fluorometrically or spectrophotometrically. The optical density (570 nm) or fluorescence intensity (lex/em=530/585 nm) is a direct measure of the enzyme activity. This reaction has been used to detect as little as 10 picomoles of H_2O_2 in a 100 µL volume (50 nM) or 1×10^{-5} U/mL of HRP.

Contents

Component	Size
Mes-Red	300µL
1X Reaction buffer	150 mL
Horseradish peroxidase	1mL, 10 U/mL *
Hydrogen peroxide (H_2O_2)	200 µL of a stabilized ~3% solution **

* 1 unit (U) is defined as the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 and 20°C.

** Actual concentration is indicated on the label.

Preparing 20 mM Hydrogen Peroxide (H_2O_2) working solution

Dilute the ~3% H_2O_2 into the appropriate volume of 1X Reaction Buffer. The actual concentration of H_2O_2 is indicated on the label. For instance, you can prepare a 20 mM H_2O_2 working solution from a 3.0% (0.88 M) H_2O_2 stock solution by diluting 22.7 µL of 3.0% H_2O_2 into 977 µL of 1X Reaction Buffer.

Note that although the ~3% H_2O_2 stock solution has been stabilized to slow its degradation, the 20 mM H_2O_2 working solution prepared in this step will be less stable and should be used within a few hours of preparation.

Experimental Protocols - H_2O_2 Assay

The following protocol describes the H_2O_2 assay in a total volume of 100 µL per microplate well. The volumes recommended here are sufficient for ~100 assays.

Prepare an H_2O_2 standard curve. Dilute the appropriate amount of 20 mM H_2O_2 working solution into 1X Reaction Buffer to produce H_2O_2 concentrations of 0 to 10 µM, each in a volume of 50 µL. Be sure to include a no- H_2O_2 control. Final H_2O_2 concentrations will be two-fold lower (*e.g.,* 0 to 5 µM).

If you are not using a standard curve, prepare positive and negative controls. For a positive control, dilute the 20 mM H_2O_2 working solution to 10 µM in 1X Reaction Buffer. For a negative control, use 1X Reaction Buffer without H_2O_2.

Dilute the H_2O_2-containing samples in 1X Reaction Buffer. Use a volume of 50 µL for each reaction. A variable dilution will be required depending on the total H_2O_2 present in the sample.

In the first trial, serially dilute the samples to determine the optimal amount of sample for the assay. Note that extremely high levels of H_2O_2 (*e.g.*, 100 µM, final concentration) can produce lower fluorescence than moderately high levels (*e.g.*, 25 µM), because excess H_2O_2 can oxidize the reaction product, resorufin, to nonfluorescent resazurin.

Load the samples. Pipet 50 µL of the standard curve samples, controls, and experimental
samples into individual wells of a microplate.

Prepare a working solution of 100 µM Mes-Red reagent and 0.2 U/mL HRP. Mix the following:

- 50 µL of 10 mM Mes-Red reagent stock solution
- 100 µL of 10 U/mL HRP stock solution
- 4.85 mL of 1X Reaction Buffer

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

Begin the reactions. Add 50 µL of the Mes-Red reagent/HRP working solution to each microplate well containing the standards, controls, and samples.

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.

Measure the fluorescence or absorbance. Use a microplate reader equipped for excitation in the range of 530 nm and fluorescence emission detection at 585 nm, or for absorbance at 570 nm.(For example see Figure.1)

Correct for background fluorescence or absorbance. For each point, subtract the value derived from the no- H_2O_2 control.

Experimental Protocols - Peroxidase Assay

The following protocol describes the assay of peroxidase in a total volume of 100 µL per microplate well. The volumes here are sufficient for ~100 assays.

Prepare a peroxidase standard curve. Dilute the appropriate amount of 10 U/mL HRP stock solution into 1X Reaction Buffer to produce HRP concentrations of approximately of 0 to 2 mU/mL HRP, each in a volume of 50 µL. Be sure to include a no-HRP control. Note that the HRP concentrations will be two-fold lower in the final reaction volume.

If you are not using a standard curve, prepare positive and negative controls. For a positive control, dilute the 10 U/mL HRP stock solution to 2 mU/mL in 1X Reaction Buffer. Use 1X Reaction Buffer without HRP as a negative control.

Dilute the peroxidase-containing samples in 1X Reaction Buffer. Use a volume of 50 µL for each reaction. A variable dilution will be required depending on the total peroxidase present in the sample.

In the first trial, serially dilute the samples to determine the optimal amount of sample for the assay. Note that extremely high levels of HRP (*e.g.*, 100 mU/mL, final concentration) can produce lower fluorescence than moderately high levels (*e.g.*, 1 mU/mL), because excess HRP can oxidize the reaction product, resorufin, to nonfluorescent resazurin.

Load the samples. Pipet 50 µL of standard curve samples, controls, and experimental samples into individual wells of a microplate.

Prepare a working solution of 100 µM Mes-Red reagent containing 2 mM H_2O_2. Mix the following:

- 50 µL of 10 mM Mes-Red reagent stock solution
- 500 µL of 20 mM H_2O_2 working solution
- 4.45 mL of 1X Reaction Buffer

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

Begin the reactions. Add 50 µL of the Mes-Red reagent/ H_2O_2 working solution to each microplate well containing the standards, controls, and samples.

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.

Measure the fluorescence or absorbance. Use a microplate reader equipped for excitation in the range of 530 nm and fluorescence emission detection at 585 nm, or for absorbance at 570 nm. (For example see Figure.2)

Correct for background fluorescence or absorbance. For each point, subtract the value derived from the no-HRP control.

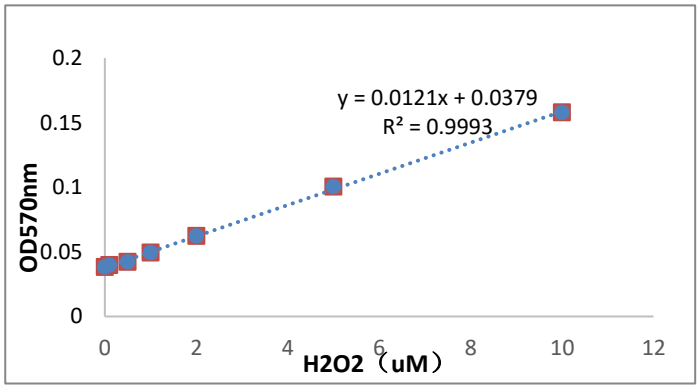

Figure .1

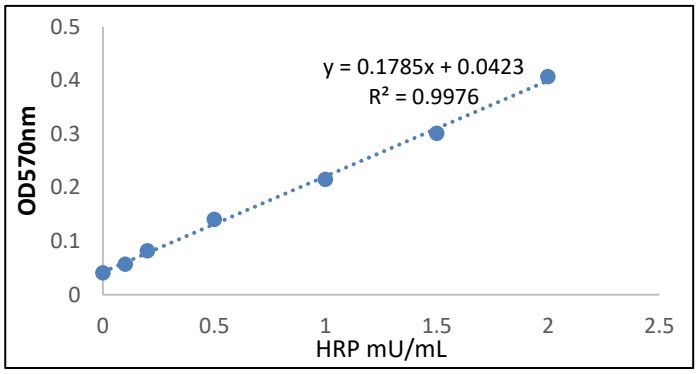

Figure .2

Storage condition

-20°C & Protected from light. Shelf life: 6 months after receipt.

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