For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

Mes-Red葡萄糖/葡萄糖氧化酶检测试剂盒
Mes-Red Glucose/Glucose Oxidase Assay Kit

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

Cat.No. MGG3839 **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

Introduction

The Mes-Red Glucose/Glucose Oxidase Assay Kit provides a sensitive one-step method for detecting glucose or glucose oxidase. The Mes-Red reagent is a colorless, stable, and extremely versatile peroxidase substrate. Because peroxidase- and glucose oxidase–mediated reactions can be coupled, it is possible to measure glucose oxidase activity or the release of glucose by any glucosidase enzyme —- for instance, β-glucosidase and glucocerebrosidase—in either a continuous or discontinuous assay. This assay should also be very useful for quantitation of glucose levels in foods, fermentation media, and bodily fluids.

In the assay, glucose oxidase reacts with d-glucose to form d-gluconolactone and H_2O_2. In the presence of horseradish peroxidase (HRP), the H_2O_2 then reacts with the Mes-Red reagent in a 1:1 stoichiometry to generate the red-fluorescent oxidation product, resorufin. Resorufin has fluorescence excitation and emission maxima of approximately 571 nm and 585 nm, respectively, and because the extinction coefficient is high (54,000 $cm^{-1}M^{-1}$), the assay can be performed either fluorometrically or spectrophotometrically. Furthermore, at these long wavelengths, there is little interference from autofluorescence found in most biological samples. With the Mes-Red Glucose/Glucose Oxidase Assay Kit, we have detected as little as 3 µM d-glucose and 0.05 mU/mL glucose oxidase.

Contents

Mes-Red reagent.	250 µL
1X Reaction Buffer	50 mL
HRP stock solution	500 µL
Glucose oxidase stock solution	500 µL
H_2O_2 solution	200 µL
400 mM glucose stock solution	6.25 mL

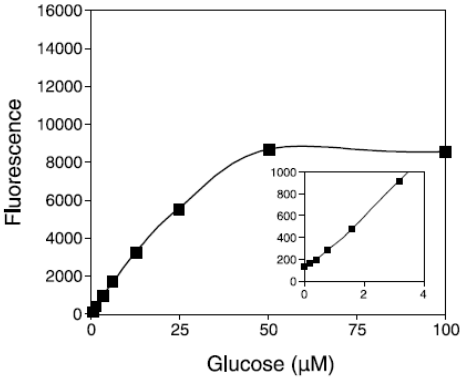

Figure 1. Detection of glucose using the Mes-Red Glucose/Glucose Oxidase Assay Kit. Reactions containing 50 µM Mes-Red reagent, 0.1 U/mL HRP, 1 U/mL glucose oxidase and the indicated amount of glucose in 50 mM sodium phosphate buffer, pH 7.4, were incubated for 37°C. Fluorescence was then measured with a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. Background fluorescence (201 arbitrary units), determined for a no–glucose control reaction, has been subtracted from each value. The inset shows the sensitivity and linearity of the assay at low levels of glucose.

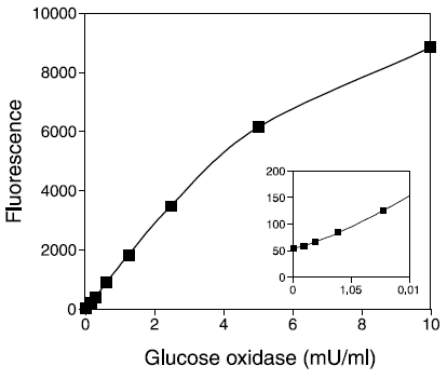

Figure 2. Detection of glucose oxidase using the Mes-Red Glucose/Glucose Oxidase Assay Kit. Reactions containing 50 µM Mes-Red reagent, 0.1 U/mL HRP, 10 mM glucose and the indicated amount of glucose oxidase in 50 mM sodium phosphate buffer, pH 7.4, were incubated for 30 minutes at 37°C. Fluorescence was measured with a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. Background fluorescence (19 arbitrary units), determined for a no–glucose oxidase control reaction, has been subtracted from each value. The inset shows the assay's sensitivity at low levels of glucose oxidase.

Experimental Protocol

The following procedure is designed for use with a fluorescence or absorbance microplate reader. For use with a standard fluorometer, volumes must be increased accordingly.

Glucose Assay

The following protocol describes the assay of glucose in a total volume of 100 µL per microplate well. The volumes recommended here are sufficient for ~100 assays. The kit provides sufficient material for ~500 assays.

1.1 Prepare a glucose standard curve.

Dilute the appropriate amount of the 400 mM glucose stock solution into 1X Reaction Buffer to produce glucose concentrations of 0 to 200 µM, each in a volume of 50 µL. Be sure to include a no-glucose control. Final glucose concentrations will be twofold lower (e.g., 0 to 100 µM).

1.2 If no standard curve is to be used, prepare positive and negative controls.

For a glucosepositive control, dilute the 400 mM glucose stock solution to 200 µM in 1X Reaction Buffer. For an H_2O_2-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.

1.3 Dilute the glucose-containing samples in 1X Reaction Buffer.

A volume of 50 µL will be used for each reaction. A variable dilution will be required depending on the total glucose present in the sample. In the first trial the samples should be serially diluted to determine the optimal amount of sample for the assay.

Note: Extremely high levels of glucose (e.g., 500 µM, final concentration) can produce lower fluorescence than moderately high levels (e.g., 100 µM), because excess H_2O_2 resulting from the reaction of glucose with glucose oxidase can oxidize the reaction product, resorufin, to nonfluorescent resazurin.

1.4 Load the samples.

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

1.5 Prepare a working solution of 100 µM Mes- Red reagent, 0.2 U/mL HRP and 2 U/mL glucose oxidase.

Mix the following:

50 µL of 10 mM Mes-Red reagent stock solution

100 µL of 10 U/mL HRP stock solution

100 µL of 100 U/mL glucose oxidase stock solution

4.75 mL of 1X Reaction Buffer

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

1.6 Begin the reactions.

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

1.7 Incubate the reactions.

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

1.8 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.

1.9 Correct for background fluorescence or absorbance.

For each point, subtract the value derived from the no-glucose control.

Glucose Oxidase Assay

The following protocol describes the assay of glucose oxidase in a total volume of 100 µL per microplate well. The volumes here are sufficient for ~100 assays. The kit provides sufficient material for ~500 assays.

2.1 Prepare a glucose oxidase standard curve.

Dilute the appropriate amount of 100 U/mL glucose oxidase stock solution into 1X Reaction Buffer to produce glucose oxidase concentrations of approximately 0 to 10 mU/mL glucose oxidase, each in a volume of 50 µL. Be sure to include a no–glucose oxidase control. Please note that final glucose oxidase concentrations will be twofold lower (e.g., 0 to 5 mU/mL).

2.2 If no standard curve is to be used, prepare positive and negative controls.

For a glucose oxidase–positive control, dilute the 100 U/mL glucose oxidase stock solution to 10 mU/mL in 1X Reaction Buffer. For an H_2O_2 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.

2.3 Dilute the glucose oxidase–containing samples in 1X Reaction Buffer.

A volume of 50 µL will be used for each reaction. A variable dilution will be required depending on the total glucose oxidase present in the sample. In the first trial, the samples should be serially diluted to determine the optimal amount of sample for the assay. Note that extremely high levels of glucose oxidase (e.g., 50 mU/mL, final concentration) can produce lower fluorescence than moderately high levels (e.g., 10 mU/mL), because excess

H_2O_2 resulting from the reaction of glucose with glucose oxidase can oxidize the reaction product, resorufin, to nonfluorescent resazurin.

2.4 Load the samples.

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

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

50 µL of 10 mM Mes-Red reagent stock solution

100 µL of 10 U/mL HRP stock solution

1.25 mL of 400 mM glucose stock solution

3.60 mL of 1X Reaction Buffer

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

2.6 Begin the reactions.

Add 50 µL of the Mes-Red reagent/HRP/glucose 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), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions.

2.8 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.

2.9 Correct for background fluorescence or absorbance.

For each point, subtract the value derived from the no–glucose oxidase control.

Mes-Red Reagent

Restrictions Please note the following restrictions on the use of the Mes-Red reagent. The Mes-Red reagent is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Mes-Red reagent is also unstable at high pH (>8.5). Furthermore, the absorption and fluorescence of the reaction product, resorufin, are pH-dependent. Below the pKa (~6.0), the absorption maximum shifts to ~480 nm and the fluorescence quantum yield is markedly lower. For these reasons, the reactions should be performed at pH 7–8. The provided reaction buffer, pH 7.4, is recommended.

Allow reagents to warm to room temperature before opening vials. The Mes-Red reagent is somewhat air sensitive. Once a vial of Mes-Red reagent is opened, the reagent should be used promptly.

Storage condition

-20°C & Protected from light.

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