For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

Mes-Red尿酸/尿酸酶检测试剂盒
Mes-Red Uric Acid/Uricase Assay Kit

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

Cat.No. MUA4597 **Size :** 400 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

Mes-Red Uric Acid/Uricase Assay Kit provides a sensitive and simple method for detecting uric acid or uricase using either a fluorescence microplate reader or fluorometer.

Serum uric acid is the end product of purine metabolism in the body tissues and is cleared through the kidneys by glomerular filtration. Most animals can metabolize uric acid to more readily excreted products, but humans lack the necessary enzyme, urate oxidase (uricase), as a result of the presence of two “nonsense mutations” in the human gene for uricase.

The Mes-Red Uric Acid/Uricase Assay Kit provides an ultrasensitive method for detecting uric acid or for monitoring uricase activity. In the assay, uricase catalyzes the conversion of uric acid to allantoin, hydrogen peroxide, and carbon dioxide. In the presence of horseradish peroxidase (HRP), hydrogen peroxide reacts stoichiometrically with the Mes-Red reagent to generate the red-fluorescent oxidation product resorufin.

Because resorufin has absorption and fluorescence emission maxima of approximately 571 nm and 585 nm, respectively, there is little interference from autofluorescence in most biological samples.

Feature

- Detect concentrations as low as 100 nM uric acid or 0.2 mU/mL of uricase activity
- Format allows for multiple time point measurements
- Designed for minimal autofluorescence interference

Contents

Mes-Red reagent	200 µL
1X Reaction Buffer	20 mL
HRP stock solution	200 µL
H_2O_2 solution	500 µL
Uricase	100 µL
Uric acid	3×1.5 mL

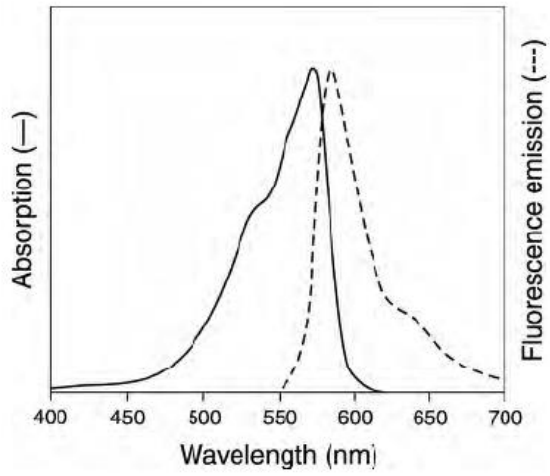

Figure 1 Detection of uric acid using the Mes-Red reagen-based assay. Each reaction contained 50 µM Mes-Red reagent, 0.2 U/mL HRP, 0.2 U/mL uricase, and the indicated amount of uric acid in 1X Reaction Buffer. After 30 minutes incubation at 37°C, fluorescence was measured in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. A background fluorescence of 26 fluorescence units was substracted from each data point.

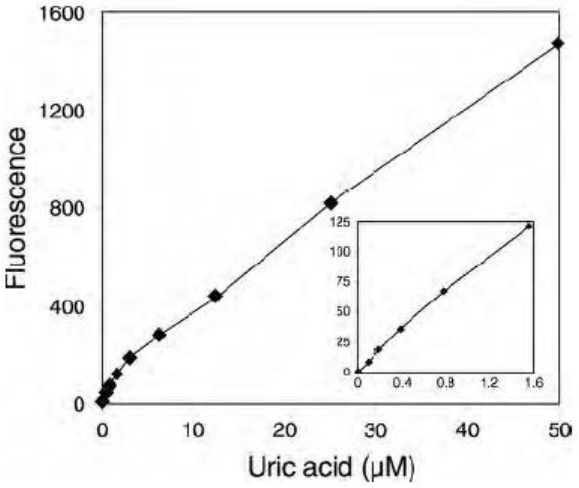

Figure 2 Detection of uric acid using the Mes-Red reagen-based assay. Each reaction contained 50 µM Mes-Red reagent, 0.2 U/mL HRP, 1 mM uric acid, and the indicated amount of uricase in 1X Reaction Buffer. After 30 minutes incubation at 37°C, fluorescence was measured in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. A background fluorescence of 26 fluorescence units was substracted from each data point.

Experimentals Protocols

The following procedure is designed for use with a fluorescence or absorbance multiwell plate scanner. For use with a standard fluorometer or spectrophotometer, volumes must be increased accordingly.

Please note that resorufin, the product of the Mes-Red reaction, is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. For this reason, the final DTT or 2-mercaptoethanol concentration in the reaction should be no higher than 10 μM.

The absorption and fluorescence of resorufin are pH-dependent. Below the pKa (~6.0), the absorption maximum shifts to ~480 nm and the fluorescence quantum yield is markedly lower. In addition, the Mes-Red reagent is unstable at high pH (>8.5). For these reasons, the reactions should be performed at pH 7–8. We recommend using the included Reaction Buffer (pH 7.5) for optimal performance of the Mes-Red reagent.

Uric Acid Assay

Tfollowing protocol describes the assay of uric acid 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 ~400 assays.

1.1 Prepare a uric acid standard curve by diluting the appropriate amount of 5 mM uric acid into 1X Reaction Buffer to produce uric acid concentrations of 0 to 100 μM. Use 1X Reaction Buffer without uric acid as a negative control. A volume of 50 μL will be used for each reaction. Note that the final concentration will be twofold lower in the final reaction.

1.2 If desired, prepare a positive control by diluting the 20 mM H_2O_2 working solution (20 mM H_2O_2 working solution can be prepared from a 3.0% H_2O_2 stock solution by diluting 23 μL of 3.0% H_2O_2 into 977 μL of dH_2O) to 10 μM in 1X Reaction Buffer.

1.3 Dilute the uric acid–containing samples in 1X Reaction Buffer. A volume of 50 μL will be used for each reaction.

1.4 Pipet 50 μL of the diluted samples, standards and controls into separate wells of a microplate.

1.5 Prepare a working solution of 100 μM Mes-Red reagent containing 0.4 U/mL HRP and 0.4 U/mL uricase according to Table 1.

Mes-Red reagent	50 μL
1X Reaction Buffer	4.91 mL
HRP stock solution	20 μL
Uricase	20 μL
Total volume	5 mL

Note that the final concentration of each component will be two-fold lower in the final reaction.

1.6 Begin the reactions by adding 50 μL of the Mes-Red reagent/HRP/uricase working solution to each microplate well containing the samples and controls.

1.7 Incubate the reactions for 30 minutes or longer at 37°C, 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 in a microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm or absorbance at ~560 nm.

1.9 For each point, correct for background fluorescence or absorbance by subtracting the value derived from the no-uric acid control.

Uric Acid Assay

The following protocol describes the assay of uricase activity 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 ~400 assays.

2.1 Prepare a uricase standard curve by diluting the 100 U/mL uricase stock solution in 1X Reaction Buffer to produce uricase concentrations of 0 to 100 mU/mL. Use 1X Reaction Buffer without uricase as a negative control. A volume of 50 μL will be used for each reaction. Note that the uricase concentration will be twofold lower in the final reaction volume.

2.2 Dilute the uricase-containing samples in 1X Reaction Buffer. A volume of 50 μL will be used for each reaction.

2.3 If desired, prepare a positive control by diluting the 20 mM H_2O_2 working solution (20 mM H_2O_2 working solution can be prepared from a 3.0% H_2O_2 stock solution by diluting 23 μL of 3.0% H_2O_2 into 977 μL of dH_2O) to 10 μM in 1X Reaction Buffer.

2.4 Pipet 50 μL of the diluted uricase-containing standards, controls and samples into separate wells of a microplate.

2.5 Prepare a working solution of 100 μM Mes-Red reagent containing 0.4 U/mL HRP and 1.0 mM uric acid according to Table 2.

Mes-Red reagent	50 μL
1X Reaction Buffer	3.93 mL
HRP stock solution	20 μL
Uric acid stock solution	1 mL
Total volume	5 mL

Note that the final concentration of each component will be two-fold lower in the final reaction.

2.6 Begin the reaction by adding 50 μL of the Mes-Red reagent/HRP/uric acid working solution to each microplate well containing the samples and controls.

2.7 Incubate the reaction for 30 minutes or longer at 37°C, 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.

Storage condition

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

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