USER GUIDE

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Version 2.0

谷氨酸检测试剂盒

Glutamate Assay Kit

Store at -20° C & in the dark

Cat.No. MGU1840 **Size :** 200 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 Glutamate Assay Kit provides an ultrasensitive method for continuously detecting glutamic acid in a fluorescence microplate reader or fluorometer. In the assay, L-glutamic acid is oxidized by glutamate oxidase to produce α-ketoglutarate, NH_3 and H_2O_2. L-Alanine and L-glutamate–pyruvate transaminase are included in the reaction to regenerate L-glutamic acid by transamination of α-ketoglutarate, resulting in multiple cycles of the initial reaction and a significant amplification of the H_2O_2 produced. The hydrogen peroxide reacts with Amplex-Red in a 1:1 stoichiometry in the reaction catalyzed by horseradish peroxidase (HRP) to generate the highly fluorescent 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. In the Glutamate Assay, if the concentration of L-glutamic acid is limiting, the fluorescence increase is proportional to the initial L-glutamic acid concentration. Using the kit, one can detect L-glutamic acid in a purified system at levels as low as 40 nM in a 30 minute reaction.

Composition

Amplex-Red reagent stock solution	100μL
HRP stock solution	25μL
Hydrogen peroxide (H_2O_2, a stabilized ~3% solution [the actual concentration is indicated on the component label])	250μL
5X Reaction buffer (0.5 M Tris-HCl, pH 7.5)	10mL
L-glutamate oxidase stock solution	160μL
L-glutamate–pyruvate transaminase stock solution	50μL
L-Glutamic acid, 200mM	500μL
L-alanine stock solution, 200M	100μL

Experimental Protocols

The following procedure is designed for use with a fluorescence multi-well plate scanner. For use with a standard fluorometer, increase volumes accordingly. Note that the product of the Amplex-Red reaction is unstable in the presence of thiols such as dithiothreitol (DTT) or 2-mercaptoethanol. For this reason, the final DTT or 2-mercaptoethanol concentration in the reaction should be less 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 Amplex-Red reagent is unstable at high pH (>8.5). For these reasons, the reaction should be performed at pH 7–8, for example by using the provided reaction buffer (pH 7.5).

Stock Solution Preparation

1. Prepare a 20 mM H_2O_2 working solution by diluting the~3% H_2O_2 stock solution into the appropriate volume of dH_2O. The actual H_2O_2 concentration is indicated on the com ponent label. For instance, a 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. Note that al though the ~3% H_2O_2 stock solution has been stabilized to slow degradation, the 20 mM H_2O_2 working solution will be less stable and should be used promptly.
2. Prepare a 1X working solution of reaction buffer by adding 4 mL of 5X reaction buffer stock solution to 16 mL of deionized water (dH_2O).

Glutamic Acid Assay

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

1. Prepare a L-glutamic acid standard curve: Dilute the appropriate amount of 200 mM L-glutamic acid stock

solution into 1X reaction buffer to produce L-glutamic acid concentrations of 0 to 20 μM. Use 1X reaction buffer without L-glutamic acid as a negative control. A volume of 50 μL will be used for each reaction. Note that the L-glutamic acid concentrations will be two-fold lower in the final reaction volume.

2. Dilute the L-glutamic acid-containing samples in 1X reaction buffer. Use a volume of 50 μL for each reaction.

3. Prepare a positive control by diluting the 20 mM H_2O_2 working solution to 10 μM in 1X reaction buffer.

4. Pipet 50 μL of the diluted samples and controls into separate wells of a microplate.

5. Prepare a working solution of 100 μM Amplex-Red reagent containing 0.25 U/mL HRP, 0.08 U/mL L-glutamate oxidase, 0.5 U/mL L-glutamate–pyruvate transaminase, and 200 μM L-alanine by adding :

※ 50 μL of the Amplex-Red reagent stock solution
※ 12.5 μL of the HRP stock solution
※ 80 μL of the L-glutamate oxidase stock solution
※ 25 μL of the L-glutamate–pyruvate transaminase stock solution
※ 5 μL of the L-alanine stock solution
※ 4.83 mL of 1X reaction buffer

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

6. Begin the reactions by adding 50μL of the Amplex-Red reagent/HRP/glutamate oxidase/glutamate – pyruvate transaminase/alanine working solution to each microplate well containing the samples and controls.

7. Incubate the reactions for 30 minutes or longer at 37° C, **protected from light**. Because the assay is continuous (not terminated), fluorescence may be measured at multiple time points to follow the kinetics of the reactions.

8. Measure the fluorescence in a fluorescence microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm.

9. For each point, correct for background fluorescence by subtracting the values derived from the no–glutamic acid control.

Storage condition

-20°C, protected from light.