Fluorometric Xanthine Oxidase Assay Kit

Introduction

Xanthine oxidase (XO) is an important enzyme in the purine metabolism pathway, which can catalyze the oxidation of hypoxanthine to xanthine, and then further oxidize xanthine to uric acid, and produce hydrogen peroxide (H_2O_2). XO is widely found in a variety of organisms, such as humans, animal livers, intestines, and other tissues. In some disease states, such as liver disease, XO is released from cells into the bloodstream, so the level of XO activity in the blood can be used as a sensitive indicator to assess liver damage. Detection of XO activity is of great significance for studying the pathogenesis of related diseases and monitoring the efficacy of treatment.

Fluorometric Xanthine Oxidase Assay Kit provides an ultra-sensitive method for detecting XO activity. This kit uses Amplex Red as a fluorescent probe, and under the presence of horseradish peroxidase (HRP), Amplex Red can react with H_2O_2, to produce a pink fluorescent substance, Resorufin, whose fluorescence intensity and absorbance are proportional to the XO activity.

Compared with the traditional xanthine oxidase assay kit (WST-8), this kit is more sensitive and more flexible. The kit can be detected by both fluorometric and colorimetric methods, but the sensitivity of fluorescence detection is approximately 10-fold higher than that of colorimetric methods. Therefore, fluorescence detection can be performed with a smaller amount of sample to detect activity, and the kit has a good linearity in the activity range of 0.25-4 mU/mL when used for fluorescence detection.

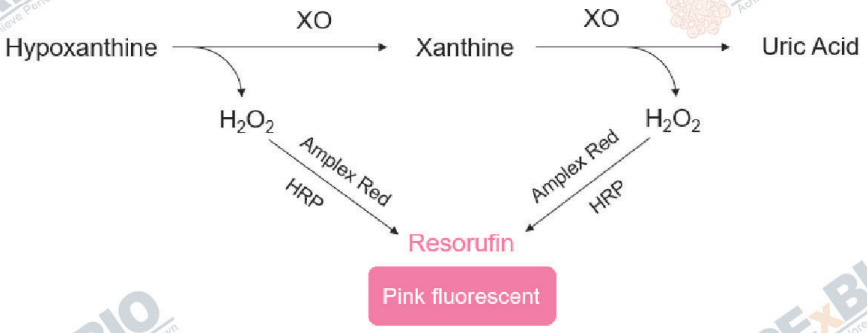

Figure 1: Xanthine oxidase assay scheme

Components and Storage

Components \ Size	100 Assays	500 Assays	Storage
5× Reaction Buffer	5 mL	25 mL	-20°C
Amplex Red Reagent	80 μL	400 μL	-20°C away from light
Horseradish Peroxidase (HRP)	50 μL	250 μL	-20°C away from light
Xanthine Oxidase	1 vial	5 vials	-20°C
Xanthine Oxidase Reconstitution Buffer	100 μL	500 μL	-20°C
Xanthine	30 μL	150 μL	-20°C
Shipping: Dry ice	Shelf life: 1 year		

Protocol

1. Warm Amplex Red Reagent and 5× Reaction Buffer to room temperature in advance. Other reagents should be placed in an ice bath for later use.

2. **Sample Preparation:**

   1) For serum samples, place whole blood at room temperature for 0.5-2 h, do not shake vigorously to avoid hemolysis, wait until the whole blood naturally coagulates and precipitate serum, centrifuge at 4ºC, 1000 g for 10 min, and take the yellow supernatant as serum sample. Store samples on ice for later use. If not assaying the same day, freeze at -80ºC for a short period, thaw and mix on ice before use.

   2) For plasma samples, collect whole blood using an anticoagulant with heparin or EDTA. Then centrifugate at 4ºC at 1000-2000 g for 10 min. pipette off the yellow or light yellow supernatant as plasma sample. Store samples on ice for later use. If not assaying the same day, freeze at -80ºC for a short period, thaw and mix on ice before use.

   3) For tissue samples: Wash the samples 1-2 times with PBS. Then Add 0.5-1 mL of lysis buffer per 1 g of tissue and homogenize on ice. After homogenization, centrifuge at 12000 g for 5-10 min at 4ºC, and take the supernatant for subsequent detection. Store samples on ice for later use. If not assaying the same day, freeze at -80ºC for a short period, thaw and mix on ice before use.

   4) For cell samples: Add 100-200 μL of lysis buffer per 10^6 cells, and lysis cells on ice for 5-10 min. Centrifuge at 12,000 g at 4ºC for 5-10 min, and take the supernatant for subsequent detection. Store samples on ice for later use. If not assaying the same day, freeze at -80ºC for a short period, thaw and mix on ice before use.

3. **1× Reaction Buffer preparation:** Dilute an appropriate amount of 5× Reaction Buffer with ddH_2O to make 1× Reaction Buffer.

4. **Amplex Red Working Solution preparation:** Refer to the table below for preparation. The Amplex Red

Working Solution can be used on the same day if stored at 4ºC or in an ice bath protected from light, but it is recommended to use it as soon as possible.

Samples	10	20	100
1× Reaction Buffer	481 μL	962 μL	4.81 mL
Amplex Red Reagent	5 μL	10 μL	50 μL
Horseradish Peroxidase (HRP)	4 μL	8 μL	40 μL
Xanthine	10 μL	20 μL	100 μL
Total Volume	500 μL	1 mL	5 mL

***Note:** The presence of hydrogen peroxide interferes with the detection of xanthine oxidase. If the sample contains hydrogen peroxide, set up the sample background control wells at the same time, i.e., use the Amplex Red Working Solution without Xanthine. The readings of the sample wells are calculated by subtracting the readings of the sample background control wells.

5. **Standard preparation:**

1) Dissolve a vial of Xanthine Oxidase in 50 μL of Xanthine Oxidase Reconstitution Buffer to make the Xanthine Oxidase (1U/mL). After use, divide the remaining Xanthine Oxidase (1U/mL) into small aliquots and store them at -20°C.

2) Dilute 2 μL of Xanthine Oxidase (1U/mL) in 198 μL of 1× Reaction Buffer to make Xanthine Oxidase (10 mU/mL). Then refer to the table below to make a series of Xanthine Oxidase standards：

Xanthine Oxidase (10 mU/mL)	1× Reaction Buffer	Final concentration (mU/mL)
0 μL	100 μL	0
2.5 μL	97.5 μL	0.25
5 μL	95 μL	0.5
10 μL	90 μL	1
20 μL	80 μL	2

6. **Add samples:**

1) Add 50 μL of sample and different concentrations of standard to the 96-well plate. If the concentration of the pre-experimental sample is outside the range of the standard curve, the sample can be diluted. The dilution should be determined according to the type of sample. For serum/plasma samples, 1× Reaction Buffer should be used for dilution. For cell or tissue lysis samples prepared with lysis buffer, lysis buffer should be used for dilution.

***Note:** The detection can be performed using colorimetric or fluorometric methods. For small sample volumes, fluorescence is preferred. If fluorescence detection is chosen, a black 96-well plate is recommended.

2) Add 50 μL of Amplex Red Working Solution per well, and mix well.

7. **Incubation:** Incubate at 37°C in the dark for 30 min. If the absorbance is low or the fluorescence is weak, the

reaction time can be extended appropriately, such as 45 or 60 minutes. In addition, since the assay is continuous (not terminated), the assay can be performed at multiple time points to track the kinetics of the reaction.

8. **Detection:** Measure the absorbance at 570 nm or fluorescence (Ex/Em: 560/590 nm).
9. **Analysis:** Establish a standard curve first, and then calculate the activity of xanthine oxidase in the sample according to the standard curve. If the signal of the background control well of the sample is relatively high, it should be subtracted to get the corrected signal. If the sample is diluted, it will need to be multiplied by the dilution factor at the end.

Note

1. Amplex Red Reagent is not stable in air and should be used as soon as possible after opening and protected from light.
2. The product of Amplex Red reaction, resorufin, is very unstable in the presence of reducing agents, so the concentration of dithiothreitol (DTT), β-mercaptoethanol, or similar reducing agent in the final reaction should be less than 10 μM.
3. The pH of the reaction should be controlled between 7 and 8, otherwise the stability of Amplex Red will be affected.
4. Amplex Red needs to be completely thawed and equilibrated to room temperature before use, otherwise the results will be affected. Other solutions should be used on ice.
5. To ensure that the sample detection value is within the standard curve, the sample can be diluted in a series of ways; It is also possible to conduct a pre-experiment to determine the approximate concentration of the sample, and then adjust the sample amount based on the results of the pre-experiment.
6. For your safety and health, please wear lab coats and gloves during the experiment.
7. For research use only. Not to be used in clinical diagnostic or clinical trials.

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