ab138885

Glutamate Oxidase Assay Kit (Fluorometric - Near Infrared)

Instructions for Use

For detecting Glutamate Oxidase in solution and cell lysates by using our proprietary red fluorescence probe

This product is for research use only and is not intended for diagnostic use.

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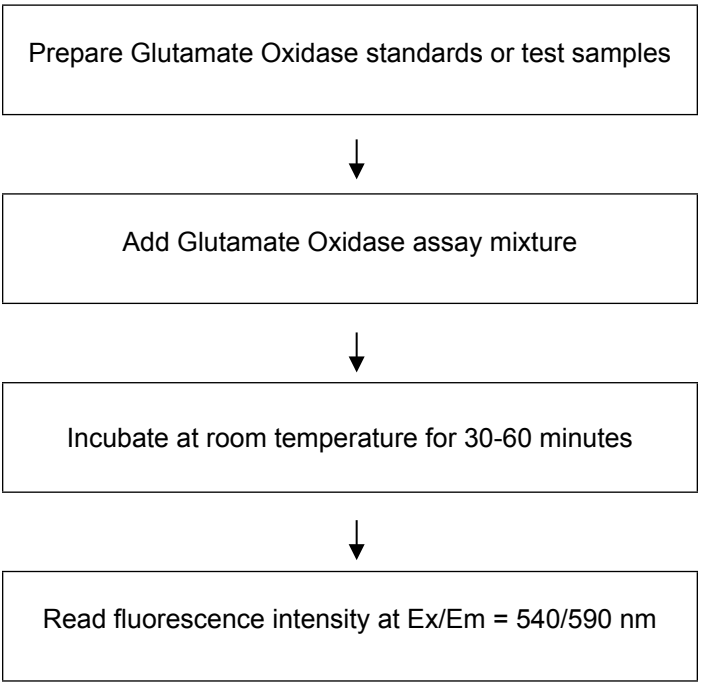
1. Introduction

Glutamate oxidase specifically catalyzes the oxidative deamination of L-glutamate in the presence of water and oxygen with the formation of ketoglutarate, ammonia, and hydrogen peroxide. Glutamate oxidase can be used as an analytic reagent and as a basis for developing biosensors for the determination of L-glutamate, L-glutamine, ammonia, and creatinine. These biosensors can be used in clinical biochemistry for the determination of glutamate-pyruvate transaminase and glutamate-oxalacetate transaminase in biological fluids, which makes the early diagnosis of heart and liver diseases possible.

ab138885 provides a quick and an ultrasensitive method for the measurement of glutamate oxidase in solution and in cell lysates. In the assay, L-glutamic acid is oxidized to α-ketoglutarate, NH_3 and H_2O_2 by glutamate oxidase. The kit uses our AbRed Indicator substrate which can react with H_2O_2 when catalyzed by horseradish peroxidase (HRP) to generate the highly fluorescent product. The signal can be read with either a fluorescence microplate reader at Ex/Em = 530-570 nm/590 -600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576±5 nm. Ab138886 can detect as little as 40 µU/mL glutamate oxidase in a 100 µL reaction volume. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: AbRed Indicator (light sensitive)	1 vial
Component B: Assay Buffer	1 x 20 mL
Component C: Horseradish Peroxidase (lyophilized)	1 vial
Component D: Glutamic Acid	3.4 mg
Component E: Glutamate Oxidase Standard (lyophilized)	1 x 15 mU
Component F: DMSO	1 x 200 µL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 96-well black wall, solid bottom or 384-well microplates
- Fluorescence microplate reader

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare Stock Solutions

1. **AbRed Indicator stock solution (250X):** Add 40 µL of DMSO (Component F) into the vial of AbRed Indicator (Component A). The stock solution should be used promptly. Any remaining solution needs to be aliquoted and refrozen at -20 °C.

   Note 1: Avoid repeated freeze-thaw cycles.
   Note 2: The AbRed Indicator 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 AbRed Indicator is also unstable at high pH (> 8.5). Therefore, the reaction should be performed at pH 7–8. The provided assay buffer (pH 7.4) is recommended.

2. **HRP stock solution (400X):** Add 200 µL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).
   Note: The unused HRP stock solution should be divided into single use aliquots and stored at -20 °C.

3. **Glutamic Acid stock solution (400X):** Add 1 mL of ddH_2O into the vial of Glutamic Acid (Component D). *Note: The unused glutamic acid stock solution should be divided into single use aliquots and stored at -20 ˚C.*

4. **Glutamate Oxidase (GO) Stock Solution (150 mU/mL):** Add 100 µL of Assay Buffer (Component B) into the vial of Glutamate Oxidase Standard (Component E).

   Note: The unused glutamate oxidase stock solution should be divided into single use aliquots and stored at -20 °C.

B. Prepare Assay Reaction Mixture

Prepare assay reaction mixture according to the following table, protect from light.

Components	Volume
AbRed Indicator stock solution (250X, from Step A.1)	20 µL
HRP (400X, from Step A.2)	12.5 µL
Glutamic Acid (400X, from Step A.3)	12.5 µL
Assay Buffer (Component B)	5 mL
Total Volume	5.07 mL

Table 1. Assay reaction mixture for one 96-well plate (2X)

C. Prepare serially diluted GO standards (0 to 10 mU/mL):

1. Add 30 µL of 150 mU/mL GO stock solution (from Step A.4) into 420 µL of Assay Buffer (Component B) to get 10 mU/mL GO standard solution.

2. Take 150 µL of 10 mU/mL GO standard solution to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.0, 1 and 0 mU/mL serially diluted GO standards.

3. Add GO standards and/or GO-containing test samples into a black wall/solid bottom 96-well microplate as described in Tables 2 and 3.

BL	BL	TS	TS .....
GO1	GO1	....	....
GO2	GO2	....	....
GO3	GO3		
GO4	GO4		
GO5	GO5		
GO6	GO6		
GO7	GO7		

Table 2. Layout of glutamate oxidase standards and test samples in a solid black 96-well microplate.
Note: GO= glutamate oxidase Standards, BL=Blank Control, TS=Test Samples.

GO Standard	Blank Control	Test Sample
Serial dilutions*: 50 µL	Assay Buffer (Component B) : 50 µL	50 µL

Table 3. Reagent composition for each well.

**Note 1: Add the serially diluted glutamate oxidase standards from 0.01 mU/mL to 10 mU/mL into wells from GO1 to GO7 in duplicate.*

**Note 2: High concentration of glutamate oxidase may cause reduced fluorescence signal due to the over oxidation of AbRed Indicator (to a non-fluorescent product).*

D. Run GO Assay:

1. Add 50 µL of assay reaction mixture (from Step B), to each well of glutamate oxidase standard, blank control, and test samples (see step B, Table 3) to make the total glutamate oxidase assay volume of 100 µL/well.

   Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

2. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.

3. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em= 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 nm), cutoff = 570 nm.

   Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576 ± 5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

7. Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with glutamate oxidase reactions. A typical glutamate oxidase standard curve is shown in Figure 1.

Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

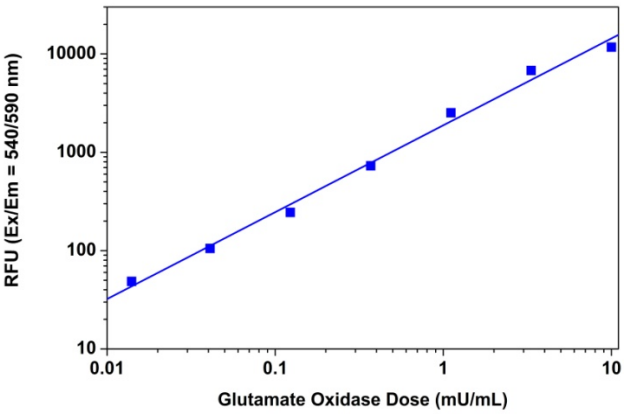


Figure 1. Glutamate oxidase dose response was measured on a 96-well black plate with ab138885. As low as 40 µU/mL glutamate oxidase can be detected with 60 minutes incubation time (n=3).

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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