Glucose Colorimetric/Fluorometric Assay Kit

Instructions for Use For quantifying Glucose oxidase in solution.

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

1. Introduction

The glucose oxidase is a dimeric protein that catalyzes the oxidation of beta-D-glucose into hydrogen peroxide and D-glucono-1,5- lactone, which is hydrolyzed to gluconic acid. It is widely used for the determination of glucose in body fluids and in removing residual glucose and oxygen from beverages, food and other agricultural products. Furthermore, Glucose oxidase is commonly used in biosensors to detect glucose.

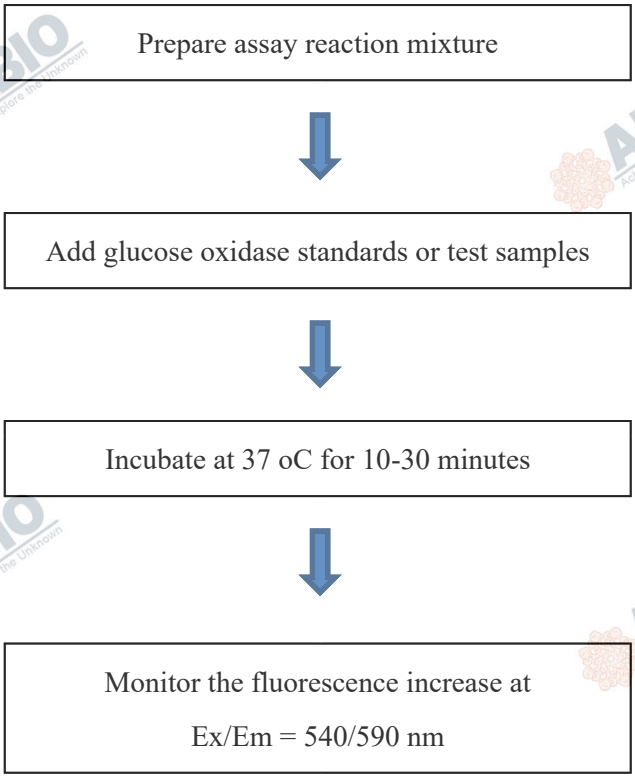
K2091 provides a quick and sensitive method for the measurement of glucose oxidase in solution. It can be performed in a convenient 96-well or 384-well microtiter plate format and readily adapted to automation without a separation step. The kit uses our AbRed Indicator which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at 576 nm. With this kit, we have detected as little as 0.05 mU/ml glucose oxidase in a 100 µl reaction volume.

Kit Key Features

- **Sensitive:** Detect as low as 0.05 mU/ml glucose oxidase in solution.
- **Continuous:** Readily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time. No wash step needed.
- **Non-Radioactive:** No special requirements for waste treatment.

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	1 vial
Component B: Assay Buffer	1 bottle (50ml)
Component C: Horseradish Peroxidase (HRP)	1 vial
Component D: Glucose Oxidase	1 vial (100 units)
Component E: DMSO	1 vial (200 µl)
Component F: Glucose	1 vial

4. Storage and Handling

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

5. Additional Materials Required

- 96 or 384-well microplates: Solid black microplates
- Fluorescence microplate reader

6. Assay Protocol

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

A. Preparation of Stock Solutions:

1. Prepare AbRed Indicator stock solution (250X) by adding 100 μl of DMSO (Component E) into the vial of AbRed Indicator (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and refrozen at -20℃.

Note: 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. Avoid repeated freeze-thaw cycles.

2. Prepare HRP stock solution (50X) by adding 1 ml of Assay Buffer (Component B) into the vial of Horseradish

Peroxidase (Component C).

Note: The unused 50X HRP stock solution should be divided into single use aliquots and stored at -20℃.

3. Prepare 100U/ml Glucose Oxidase stock solution by adding 1 ml of Assay Buffer (Component B) into the vial of Glucose Oxidase (Component D).

Note: The unused 100 U/ml glucose oxidase stock solution should be divided into single use aliquots and stored at -20℃.

4. Prepare 10X Glucose stock solution by adding 5 ml of Assay Buffer (Component B) into the vial of Glucose (Component F).

Note: The unused 10X glucose stock solution should be stored at -20℃.

B. Preparation of Assay Reaction Mixture:

Prepare assay reaction mixture according to the following tables, protected from light.

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

Components	Volume
250X AbRed Indicator	20 µl
50X HRP Stock Solution	100 µl
10X Glucose Stock Solution	500 µl
Assay Buffer (Component B)	4.3 ml
Total volume	5 ml

Table 2 Layout of glucose oxidase standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	....	....						
GOS1	GOS1	....	....	....	....						
GOS2	GOS2										
GOS3	GOS3										
GOS4	GOS4										
GOS5	GOS5										
GOS6	GOS6										
GOS7	GOS7										

Note: GOS = Glucose Oxidase Standards, BL = Blank Control, TS = Test Samples.

Table 3. Reagent composition for each well

Glucose Oxidase Standards	Blank Control	Test Sample
Serial Dilutions*: 50 µl	Assay Buffer (Component B): 50 µl	50 µl

**Note 1: Add the serially diluted glucose oxidase standards from 0.01 to 10 mU/ml into each well from GOS1 to GOS7 in duplicate.*

Note 2: High concentration of glucose oxidase (e.g., 100 mU/ml, final concentration) may cause reduced fluorescence signal due to the overoxidation of AbRed Indicator (to a non-fluorescent product).

C. Run Glucose Oxidase assay:

1. Prepare a glucose oxidase standard by diluting 2 µl of the 100 U/ml glucose oxidase stock solution into 200 µl of Assay Buffer (Component B) to have 1000 mU/ml glucose oxidase standard solution. And then

take 10 µl of 1000 mU/ml glucose oxidase standard solution to perform 1:100 and 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 mU/ml serially diluted glucose oxidase standards (50 µl/well). A non-glucose oxidase buffer is included as blank control. The final glucose oxidase concentrations should be twofold lower (i.e. 0 to 5 mU/ml).

2. Add 50 µl of assay reaction mixture into each well of glucose oxidase standards, blank control, and test samples to make the total glucose 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.*

3. Incubate the reaction for 10 to 30 minutes at 37℃, protected from light.

4. Monitor the fluorescence increase by using a fluorescence plate reader at Ex/Em = 530-570/590-600 nm (optimal Ex/Em = 540/590 nm).

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

7. Data Analysis

The fluorescence in blank wells (with the dilution buffer only) is used as a control, and is subtracted from the values for those wells with the glucoase oxidase reaction.

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.

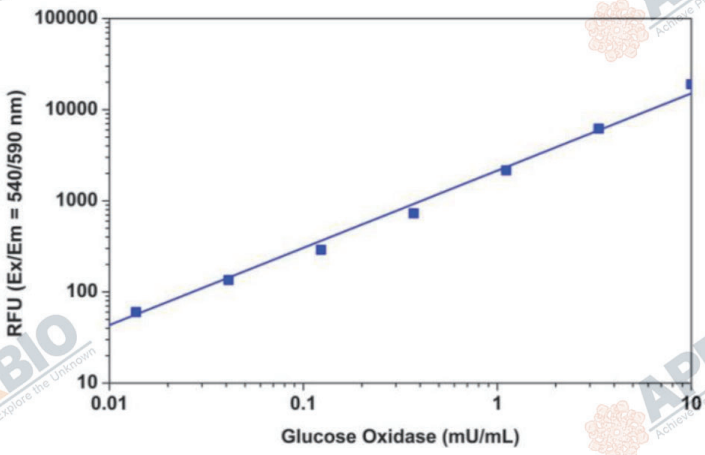


Figure 1. Glucose oxidase dose response was measured with K2091 in a solid black 96-well plate using a microplate reader. As low as 0.05 mU/ ml glucose oxidase was detected with 30 minutes incubation (n=3).

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
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*** or **Deproteinizing sample preparation kit**
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freezethaw 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 volumes not to pipette too small
	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 research use only! Not to be used in humans.

For more details, please visit http://www.apexbt.com/or contact our technical team.