CheKine™ Glucose Oxidase Activity Assay Kit

Item NO.	Product Name
KTB1310	CheKine™ Glucose Oxidase Activity Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201905
Abbkine, Inc • China • ***www.abbkine.com***

TABLE OF CONTENTS

INTRODUCTION

Background

The glucose oxidase enzyme (GOx) (EC 1.1.3.4) is a dimeric enzyme that catalyzes oxidation of beta-D-glucose into hydrogen peroxide and D-glucono-1,5-lactone, which is hydrolyzed to gluconic acid. This enzyme is produced by certain species of insects, fungi, and bacteria. Glucose oxidase 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.

Assay principle

CheKine™ Glucose Oxidase Activity Assay Kit provides a simple and easy colorimetric assay for detecting Glucose Oxidase Activity in biological samples such as in serum or plasma, cells, urine, other body fluid, food, growth medium. In this assay, GOD activity is determined by a coupled enzyme assay, in which GOD oxidizes D-glucose resulting in the production of hydrogen peroxide (H_2O_2) that reacts with chromogen in an acid condition, produce a product that can be measured at OD 580 nm. Therefore, the glucose oxidase activity present in the sample is proportional to the signal obtained.

Detection Range

The kit detection can reach 0.05 U/L, within range of 0.05 - 2 U/L.

Storage/Stability

Storage at –20°C and keep from light. Kit has a storage time of 12 months from receipt.

Assay Restrictions

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity			Storage conditions
	48T	96T	480T	
Assay Buffer (20x)	5 mL	10 mL	50 mL	4ºC
Glucose (0.2 M)	3 mL	6 mL	30 mL	-20ºC
H_2O_2 Standard (0.88 M)	100 µL	100 µL	100 µL	-20ºC, protect from light
Chromogen	2.5 mL	5 mL	25 mL	-20ºC, protect from light
Glucose Oxidase (control)	100 µL	100 µL	100 µL	-20ºC, protect from light

Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 580 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Reagent preparation

Assay Buffer (20x): Equilibrate to room temperature before use. Make a 1:20 dilution of the concentrated Assay Buffer with distilled or deionized water. This final Assay Buffer should be used to dilute the H_2O_2 standards and samples prior to assay. When stored at 4°C, this diluted Assay Buffer is stable for at least two months.
Glucose (0.2M): Ready to use as supplied. Equilibrate to room temperature during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
H_2O_2 Standard (0.88M): Ready to use as supplied. Equilibrate to room temperature protected from light during the assay. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
Chromogen: Ready to use as supplied. Equilibrate to room temperature protected from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
Glucose Oxidase (control): The vial contains Glucose Oxidase from Aspergillus niger and is used as a positive control. Take 2 µl of the Glucose Oxidase and dilute with 998 µL of Assay Buffer. A 50 µl of this diluted enzyme per well causes a markedly absorbance. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.

Standard preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours

Prepare 10 mM of H_2O_2 Standard by diluting 10 µL 0.88 M H_2O_2 Standard into 870 µL Assay Buffer. Prepare 40 µM of H_2O_2 Standard by diluting 4 µL 10mM H_2O_2 Standard into 996 µL Assay Buffer. Using 40 µM standard, prepare standard curve dilution as described in the table in microcentrifuge tubes:

	Volume of 40 µM Standard	Assay Buffer (1x)	Concentration
Std.1	200 µL	0 µL	40 µM
Std.2	150 µL	50 µL	30 µM
Std.3	100 µL	100 µL	20 µM
Std.4	50 µL	150 µL	10 µM
Std.5	25 µL	175 µL	5 µM
Std.6	10 µL	190 µL	2 µM
Std.7	5 µL	195 µL	1 µM
Blank	0	200 µL	0

Sample Preparation

Note: We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month.

Tissue samples: Perfuse tissue with cold PBS to remove any red blood cells. Homogenize tissue at 1 mL/0.1 g in cold Assay Buffer (1x). Centrifuge for 5 minutes at 4°C, 12,000 x g to get the supernate.

Cell and bacteria: Harvest the amount of cells (adherent/suspension) or bacteria necessary for each assay (initial recommendation = 1 x 10^6 cells/assay). Wash cells with cold PBS. Homogenize cells (1×10^6) with 200 µL of ice-cold Assay Buffer (1x). Centrifuge for 5 minutes at 4°C, 12,000 x g to remove any insoluble material. Collect supernatant and transfer to a new tube.

Plasma, Serum, Urine (and other biological fluids): Tested directly by adding samples to microcentrifuge tubes. However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the samples.

Assay procedure

1. Reaction. Transfer 50 µL diluted standards and samples to appropriately labeled tubes. Transfer 50 µL 0.2 M Glucose to each tube. Close the tubes tightly and mix. Place the tubes in a tube holder and incubate for 20 min at 37°C.
2. Transfer 60 µL into a clear bottom 96-well plate. Add 40 µL of Chromogen per well quickly. Tap plate to mix briefly and thoroughly. Immediately read optical density at 580nm (OD_0). Incubate for 10 min at 37°C in the dark. Read optical density at 580nm again (OD_{10}).

DATA ANALYSIS

Calculation of results

1. For each standard and sample well, calculate $\Delta OD = OD_{10} - OD_0$.
2. Subtract blank ΔOD from the standard ΔOD values and plot the ΔOD against standard concentrations. Determine the slope using linear regression fitting. Calculate the activity using the equation below:

$$\text{GO Activity} = \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{\text{Slope} \times t} \times n\ (\text{U/L})$$

ΔOD_{SAMPLE} and ΔOD_{Blank}: the change in optical density of the sample and blank, respectively.
Slope: the slope of the H_2O_2 standard curve
t: the reaction time (20 minutes)
n: the dilution factor.
Notes: If the calculated sample glucose concentration is higher than 2 U/L, dilute sample in Assay Buffer (1x) and repeat the assay. Multiply result by the dilution factor (n). For samples with low Glucose Oxidase activity, the reaction time can be increased.
Unit definition: 1 U/L of Glucose Oxidase catalyzes 1 µmole of H_2O_2 per minute at pH 5.1 at 37°C.

Typical data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.