CREATIVE BIOMART®
Assay Kit

Glucokinase assay kit

Product Information

Cat

Kit-2410

Cat.No.

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Product Overview

Glucokinase mediates phosphorylation of glucose. Allosteric agents which could activate GK activities or increase GK affinity to glucose will be a useful lead molecular to develop novel therapeutics for anti-diabetes. Glucokinase kit is a convenient, high-throughput method for enzyme-luminescence detection of glucokinase activity, which can be used for screening of glucokinase modulators.

Description

Glucokinase (GK, hexokinae IV, D) mediates phosphorylation of glucose at the sixth carbon position in the initiation step of glycolysis and glycogen synthesis in the liver. GK plays central role in blood glucose homeostasis and has low affinity to glucose in physiological conditions. The increased glucose level induces the conformational change of GK from inactive form to active form to keep glucose homeostasis. Allosteric agents which could activate GK activities or increase GK affinity to glucose will be a useful lead molecules to develop novel therapeutics for anti-diabetes. Recently, several GK activating agents were reported as a candidates for anti-diabetic drugs. This user-friendly and highly sensitive glucokinase assay kit is a useful tools to explorer novel glucokinase modulators for anti-diabetes drug development. This luminescent system has several advantages such as non-radioactive and homogeneous, no requirement of specific antibodies, short hand-on times, cost-effective HTS assay system over other assay systems.
Kinase reaction: Substrate (glucose) + ATP --> Phospho- substrate + ADP
Signal detection: Luciferin + ATP +1/2 O2 --> Oxyluciferin + AMP +CO2+PPi

Storage

All components may be stored at –80°C. If unable to do so, store liquid Glucokinase (Component A)

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Glucokinase assay kit

and Control GK activator (Component C) at –80°C and all other components at –20°C until use. Properly stored products will be active for at least 6-12 months.

Size

100 Assays

Kit Components

Comp A. Glucokinase (Liver type)
Comp B. Assay buffer
Comp C. Control GK activator
Comp D. Detection reagent
Comp E. Deionized water
White microplate

Materials Required but Not Supplied

Luminescent plate reader: capable of emission at 560 ±10 nm
Multichannel pipettor or liquid handling device
Microcentrifuge

Assay Protocol

Example #1: High throughput assay of Glucokinase modulators
1. Turn on plate reader about 30 min before use and set up plate reader to record "glow type" to get high signal-to-noise ratio with emission at 560 ± 10 nm, integration time of 250~1000msec and incubation temperature at 25°C.
2. Set up microplate format as Greiner bio-one's 96-well or 384-well plate by choosing microplate format in your plate reader program or manually.
3. Prepare solutions, set up reactions and data collections. Before opening tubes, briefly spin in order for solution to collect at the bottom. To test your machine you can prepare several assays. For example, prepare solutions for 11 assays and run 10 assays (5 assays with assay buffer only, 5 assays with provided kinase and substrate in master mix table of the following page) in a proper microplate.
4. Preparation solutions:

Glucokinase assay kit

For test compound and control activator (Comp C): Prepare test compound in DMSO or proper vehicle. Deliver test compound, control activator or solvent into triplicates of wells up to 1 ul/well in a 96-well microplate or 0.2 ul/well in a 384-well plate.
5. Whole procedures are consisted of a kinase reaction and a signal detection reaction. Kinase reaction: Prepare master mix as an example table below. For 96-wll or 384-well microplates, kinase reactions/well are recommended 50ul or 10ul, irrespectively. For kinase reaction, deliver 50ul or 10ul of the prepared master mix into each wells containing test compound, solvent, control GK activator (Comp C) and incubate plate at 25°C for 1hr with proper seal. For Signal detection: Following kinase reaction, add equal amount of detection reagent (Comp D) into each wells to stop kinase reaction, gently mix and collect signal at 560nm at 25°C.

Master mix	96-well format (50ul / well / assay)	384-well format (10ul / well / assay)
Assay buffer (B)	50 ul x 100 assays = 5000 ul	10 ul x 400 assays = 4000 ul
L-Glucokinase (A)	0.2ul x 100 assays = 20 ul	0.04 ul x 400 assays = 16 ul
Total	5020 ul	4016 ul

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