CREATIVE BIOMART®
Assay Kit

Glucose Uptake Assay Kit (bioluminescent)

Product Information

Cat.No.

Kit-2525

Product Overview

The Glucose Uptake Assay Kit is a non-radioactive, plate-based, homogeneous bioluminescent method for measuring glucose uptake in mammalian cells based on the detection of 2-deoxyglucose-6-phosphate (2DG6P). When 2-deoxyglucose (2DG) is added to cells, it is transported across the membrane and rapidly phosphorylated in the same manner as glucose. However, enzymes that further modify glucose-6-phosphate (G6P) cannot modify 2DG6P, and thus this membrane-impermeable analyte accumulates in the cell. After a brief period of incubation, an acid detergent solution (Stop Buffer) is added to lyse cells, terminate uptake and destroy any NADPH within the cells. A high-pH buffer solution (Neutralization Buffer) is then added to neutralize the acid. A Detection Reagent containing glucose-6-phosphate dehydrogenase (G6PDH), NADP+, Reductase, Ultra-Glo™ Recombinant Luciferase and proluciferin substrate is added to the sample wells. G6PDH oxidizes 2DG6P to 6-phosphodeoxygluconate and simultaneously reduces NADP+ to NADPH. The Reductase uses NADPH to convert the proluciferin to luciferin, which is then used by Recombinant Luciferase to produce a luminescent signal that is proportional to the concentration of 2DG6P.

Size

50 reactions

Kit Components

The system contains sufficient reagents to perform 50 reactions in 96-well plates (50μl of sample + 25μl of Stop Buffer + 25μl of Neutralization Buffer + 100μl of 2DG6P Detection Reagent). Includes:

- 5ml Luciferase Reagent
- 15ml Stop Buffer
- 15ml Neutralization Buffer
- 50μl NADP+ (20mM)

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• 125μl Glucose-6-Phosphate Dehydrogenase (G6PDH)
• 25μl Reductase
• 55μl Reductase Substrate
• 250μl 2-deoxyglucose (2DG, 100mM)
• 50μl 2DG6P Standard (1mM)

Materials Required but Not Supplied

• phosphate-buffered saline (PBS, e.g., Sigma Cat.# D8537 or Gibco Cat.# 14190) or other cell compatible glucose-free buffer
• 96-well assay plates (white or clear bottom, e.g., Corning Cat.# 3903)
• luminometer (e.g., GloMax® Discover Cat.# GM3000)
Note: Be sure to prepare 2DG6P Detection Reagent 1 hour before use to minimize assay background.

Preparation

Reagent Preparation
1. Thaw all components in a room temperature water bath. Once thawed, keep the Luciferase Reagent, Stop Buffer, and Neutralization Buffer at room temperature; all other components should be placed on ice.
Note: Be sure to mix thawed components to ensure homogenous solutions prior to use.
2. To prepare the 2DG6P Detection Reagent, add components to the Luciferase Reagent in the relative volumes listed below.
Component Per Reaction Per 10ml
Luciferase Reagent 100μl 10ml
NADP+ 1μl 100μl
G6PDH 2.5μl 250μl
Reductase 0.5μl 50μl
Reductase Substrate 0.0625μl 6.25μl
Note: After preparation, allow the 2DGP Detection Reagent to equilibrate at room temperature for 1 hour before use to minimize assay background.
3. Dilute 2-deoxyglucose from 100mM to 1mM in PBS or other cell compatible glucose-free buffer.

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4. Components can be refrozen, but the 2DG6P Detection Reagent should be used on the day it is prepared. We recommend to prepare only the amount of reagent needed per assay.

Assay Protocol

1. Treat cells as desired. Remove medium and wash with 100μl PBS if glucose is present.
Note: To most efficiently remove glucose from the cell culture, we recommend slow removal of the medium and the PBS using a pipettor.
2. Add 50μl of the prepared 1mM 2DG per well, shake briefly, and incubate 10 minutes at room temperature. The optimal number of cells and incubation time will vary with different cell types. If the medium does not contain glucose, a concentrated aliquot (above 1mM) of 2DG can be added directly to the cells without medium removal (e.g., 5μl of 10mM 2DG to a 50μl sample).
3. Add 25μl of Stop Buffer and shake briefly.
4. Add 25μl of Neutralization Buffer and shake briefly.
5. Add 100μl of 2DG6P Detection Reagent and shake briefly. If fewer dispensing steps are desired, the Neutralization Buffer may be added to the 2DG6P Detection Reagent just prior to assay, and the combination can be added in a volume of 125μl.
Note: Be sure to prepare 2DG6P Detection Reagent 1 hour before use to minimize assay background.
6. Incubate for 0.5–5 hours at room temperature.
7. Record luminescence using a 0.3–1 second integration on a luminometer.

Assay Controls
A number of control reactions can be performed to measure the background of the assay. Control reactions can confirm that the assay is generating a net signal above background. If the net signal is comparable to the background, the background can be subtracted from the signal to more correctly represent fold changes under given experimental conditions.
Each of the recommendations listed below create conditions where no 2DG6P accumulates.
1. Omit 2DG: Adding PBS without 2DG provides no substrate for transport.
2. Add the Stop Buffer prior to 2DG: The acidic detergent solution can disrupt membranes and inactivate kinases before addition of 2DG, so no 2DG6P can be produced.

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3. Incubate cells with a glucose transporter inhibitor: Adding an inhibitor (e.g., 50μM cytochalasin B for 5 minutes) before performing the assay prevents transport of 2DG inside the cells.

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