

Product Information

Glucose Uptake Colorimetric Assay Kit

Catalog Number **MAK083**Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

Glucose is the primary source of energy for most cells and its uptake into cells is highly regulated and the first rate limiting step in glucose metabolism. Glucose uptake is facilitated by the GLUT family of transporter proteins, whose expression and activity are regulated by multiple mechanisms. Glucose uptake is upregulated in many cancer cells, which exhibit high rates of aerobic glycolysis. Cells exhibiting insulin resistance show diminished glucose uptake in response to insulin stimulation.

The Glucose Uptake Colorimetric Assay kit provides a simple and direct procedure for measuring glucose uptake in a variety of cells. Glucose uptake is measured using the glucose analog, 2-deoxyglucose (2-DG), which is taken up by cells and phosphorylated by hexokinase to 2-DG6P. 2-DG6P cannot be further metabolized and accumulates in cells, directly proportional to the glucose uptake by cells. In this assay, 2-DG uptake is determined by a coupled enzymatic assay in which the 2-DG6P is oxidized, resulting in the generation of NADPH, which is then determined by a recycling amplification reaction in which the NADPH is utilized by glutathione reductase in a coupled enzymatic reaction that produces glutathione. Glutathione reacts with DTNB to product TNB, which is detected at 412 nm.

The use of the recycling amplification reaction in the colorimetric assay results in the **limit of detection** being **10-fold lower** (20–100 pmole) **compared to the fluorescence assay** (200–1,000 pmole, MAK084).

Components

The kit is sufficient for 100 assays in 96 well plates.

Extraction Buffer 17 mL
Catalog Number MAK083A

Neutralization Buffer 2.5 mL
Catalog Number MAK083B

2-Deoxyglucose (2-DG), 10 mM 1 mL
Catalog Number MAK083C

Assay Buffer 25 mL
Catalog Number MAK083D

Enzyme Mix 1 vL
Catalog Number MAK083E

Recycling Mix 1 vL
Catalog Number MAK083F

2-DGCP Standard 1 vL
Catalog Number MAK083G

Glutathione Reductase 2 vL
Catalog Number MAK083H

Substrate-DTNB 2 vL
Catalog Number MAK083I

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Krebs-Ringer-Phosphate-HEPES (KRPH) Buffer – 20 mM HEPES, 5 mM KH_2PO_4 , 1 mM MgSO_4 , 1 mM CaCl_2 , 136 mM NaCl, and 4.7 mM KCl, pH 7.4

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Warm all buffers to room temperature before use.

Enzyme Mix and Recycling Mix – Reconstitute each with 220 μL of Assay Buffer. Mix well by pipetting (do not vortex), then aliquot each and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution and keep cold while in use.

2-DG6P Standard – Reconstitute with 100 μL of water to generate a 10 mM (10 nmole/ μL) 2-DG6P Standard solution. Mix well by pipetting, then aliquot and store, protected from light, at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution and keep cold while in use.

Glutathione Reductase – Reconstitute with 1.1 mL of Assay Buffer. Mix well by pipetting, then aliquot and store at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution.

Substrate-DTNB – Reconstitute with 1 mL of Assay Buffer. Mix well by pipetting, then aliquot and store at $-20\text{ }^{\circ}\text{C}$. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at $-20\text{ }^{\circ}\text{C}$, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

2-DG6P Standards for Colorimetric Detection

Dilute 10 μL of the 10 mM 2-DG6P Standard solution with 990 μL of Assay Buffer to prepare a 0.1 mM (100 pmole/ μL) standard solution. Further dilute the solution to 0.01 mM (10 pmole/ μL) by adding 50 μL of 0.1 mM 2-DG6P solution to 450 μL of Assay Buffer. Add 0, 2, 4, 6, 8, and 10 μL of the 0.01 mM standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add Assay Buffer to each well to bring the volume to 50 μL .

Sample Preparation

The following cell treatment procedure is presented as a guideline only. For other cell types, optimal incubation and treatment procedures may vary. 3T3-L1 cells were seeded at 1,500 cells/well in a 96 well plate, differentiated to mature adipocytes, and maintained for an additional 4 days. Cells were then washed twice with PBS and starved in 100 μL of serum-free medium overnight. Cells were then washed 3 times with PBS and then glucose-starved by plating with 100 μL of KRPH buffer containing 2% BSA for 40 minutes. Cells were then stimulated with or without insulin (1 μM) for 20 minutes. Add 10 μL of 10 mM 2-DG, mix, and incubate for 20 minutes.

For samples, prepare a negative control by incubating a parallel sample without insulin and 2-DG.

Following incubation, wash cells 3 times with PBS. Lyse cells with 80 μL of Extraction buffer. Freeze/thaw cells in dry ice/ethanol bath or liquid nitrogen, and then heat at $85\text{ }^{\circ}\text{C}$ for 40 minutes. Cool cell lysate on ice for 5 minutes and then neutralize by adding 10 μL of Neutralization Buffer. Briefly spin down at $13,000 \times g$ to remove insoluble material and then dilute the lysate 10-fold by adding 45 μL Assay Buffer to 5 μL of sample lysate. Add 1–50 μL of sample per well and adjust final volume to 50 μL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction

1. Set up Reaction Mix A for NADPH generation according to the scheme in Table 1. 10 μL of Reaction Mix A is required for each reaction (well).

Table 1.
Reaction Mix A

Reagent	Volume
Assay Buffer	8 μL
Enzyme Mix	2 μL

2. Add 10 μL of Reaction Mix A to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Cover the plate and protect from light during the incubation.
3. To degrade NADP, add 90 μL of Extraction Buffer to each well. Seal the plate well and heat at 90 °C for 40 minutes. Cool on ice for 5 minutes and then add 12 μL of Neutralization Buffer.
4. Set up Reaction Mix B for the recycling amplification reaction according to the scheme in Table 2. 38 μL of Reaction Mix B is required for each reaction (well).

Table 2.
Reaction Mix B

Reagent	Volume
Glutathione Reductase	20 μL
Substrate-DTNB	16 μL
Recycling Mix	2 μL

5. Add 38 μL of Reaction Mix B to each of the wells. Mix well using a horizontal shaker or by pipetting.
6. Measure the absorbance at 412 nm (A_{412}) every 5 minutes in a microplate reader maintained at 37 °C. Take endpoint readings of all samples and standards when the 100 pmole standard reaches 1.5-2.0 OD.

Results

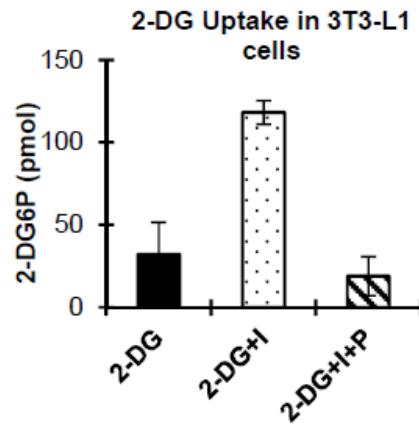
Calculations

The background for the assay is the value obtained for the 0 (blank) 2-DG6P standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate 2-DG6P standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the negative control (insulin-free) value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of accumulated 2-DG6P present in the samples, which is proportional to the amount of 2-DG in the test samples, may be determined from the standard curve.

Figure 1.
2-DG Uptake in 3T3-L1 cells



I – Insulin, activates glucose transporter
P – Phloretin, inhibits transport

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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