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Product Information

Glucose Uptake Fluorometric Assay Kit

Catalog Number **MAK084**
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 Fluorometric 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 reacts with the probe to generate a fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the 2-DG taken up by the cell.

Components

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

Extraction Buffer 17 mL
Catalog Number MAK084A

Neutralization Buffer 1 mL
Catalog Number MAK084B

2-Deoxyglucose, 10 mM 1 mL
Catalog Number MAK084C

2-DG Uptake Assay Buffer 10 mL
Catalog Number MAK084D

Enzyme Mix 1 vl
Catalog Number MAK084E

Probe 0.2 mL
Catalog Number MAK084F

2-DGCP Standard 1 vl
Catalog Number MAK084G

Reagents and Equipment Required but Not Provided.

- Two 96 well flat-bottom plates – It is recommended to use black plates with clear bottoms for fluorescence assays
- Fluorescence 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 and the probe to room temperature before use.

Enzyme Mix – Reconstitute with 220 µL of 2-DG Uptake Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °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 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

2-DG6P Standards for Fluorometric 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. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 mM standard solution into a 96 well plate, generating 0 (blank), 200, 400, 600, 800, and 1,000 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 °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 × *g* to remove insoluble material. Add up to 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 the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
2-DG Uptake Assay Buffer	47 µL
Probe	2 µL
Enzyme Mix	1 µL

2. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 40 minutes at 37 °C. Cover the plate and protect from light during the incubation.
   Note: If samples have low glucose uptake (2-DG uptake less than 100 pmole), repeat the assay and reduce the probe volume to 0.5 µL per well to reduce reagent background. Adjust the Assay Buffer accordingly.

3. Measure fluorescence intensity at λ_{ex} = 535/ λ_{em} = 587 nm.

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 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.

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 fluorometric assays, use black plates with clear bottoms
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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