Glucose Uptake Assay Kit III (FC/FM)

Easy method to image and accurately measure glucose uptake in cultured cells via FACS and fluorescence microscopy

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K682
Description	Glucose is a ubiquitous energy source in most organisms and plays a pivotal role in cellular metabolisms and homeostasis. Cancer cells exhibit increased glucose uptake to support their high proliferation rate. 2-NBDG (2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose) is a fluorescent deoxyglucose analog that can be taken up by cells through glucose transporters. However, 2-NBDG cannot be fully utilized in glycolysis because of its modification and thus accumulates inside the cells. Fluorescence generated by this fluorescent glucose analog is proportional to glucose uptake by the cells and can be used to measure glucose uptake using fluorescent microscopy and flow cytometry. To validate the assay, the kit includes phloretin, a natural phenol that inhibits glucose uptake. This easy to use non-radioactive kit allows imaging and accurate measurement of glucose uptake in cultured cells in response to insulin, growth factors cytokines, mitogens and nutrients, etc.
Quantity	50 assays
Applications	• Measurement of glucose uptake in response to insulin, growth factors, cytokines, etc. • Analysis of glucose metabolism and cell signaling in various cell types • Screening anti-diabetic drugs
Sample Type	• Cell culture: Adherent or suspension cells

Components	Quantity	Color Code
Analysis Buffer (50x)	1.8 ml	Brown
2-NBDG Reagent (100x)	200 µl	Red
Glucose Uptake Enhancer	1 ml	Blue
Phloretin (100X)	75 µl	Yellow

User Supplied Reagents & Equipment	• Cell culture medium, PBS • 24 or 12-well tissue culture plate • Fluorescence microscope • Flow cytometer with excitation filter at 488 nm wavelength
Storage and Reagents Preparation	Store kit at –20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. • **Analysis Buffer (50X):** Dilute Analysis Buffer with 1X PBS (not supplied) to make 1X Analysis Buffer. Keep on ice while in use. • **2-NBDG Reagent (100X):** Aliquot and store at -20^{0}C. For consistent results, avoid repeated freeze/thaw.
Assay Protocol	The protocol described below is for 24-well tissue culture plate. Reagents, buffer, and the number of cells to be seeded should be adjusted for different size culture plates. **1. Sample Preparation**: Seed adherent cells (2-5x10^4 cells/well) one day before starting the assay. After 8-12 hours, remove regular culture medium (10% FBS) and treat cells with test compound or vehicle control in 400 µl cell culture medium with 0.5% FBS. Incubate cells at 37^{0}C with 5% CO_2 for 1 hour or desired time period depending upon the test compound. To use Phloretin as a control, treat cells with 4 µl Phloretin (final concentration 1X) in 400 µl of cell culture medium with 0.5% FBS at 37^{0}C with 5% CO_2 for 1 hour. Note: Cell seeding is not required for suspension cells. Use up to 1-2x10^5 suspension cells/well in 400 µl of cell culture medium with 0.5% FBS to treat with test compound or vehicle control. **2. Glucose Uptake**: Prepare 400 µl glucose uptake mix for each well as following: 376 µl Cell culture medium (0.5% FBS) 4 µl 2-NBDG Reagent 20 µl Glucose Uptake Enhancer Test compound or 1X Phloretin: same concentration as in step 1

	Mix well. After the incubation in Step 1, spin down the plate at 400 x g for 5 minutes and carefully remove the medium without disturbing cells. Gently add glucose uptake mix to each well and incubate cells at 37^{0}C with 5% CO_2 for 30 minutes. **3. Measurement**: After incubation, collect cells from the plate and keep on ice. Wash once with 1 ml ice-cold 1X Analysis Buffer. Spin down at 400 x g for 5 minutes and resuspend cell pellet in 400 µl of 1X Analysis Buffer. Cells are ready to be analyzed on flow cytometer (488 nm excitation laser). For flow acquisition and analysis, select the main cell population in the FSC vs SSC plot to exclude dead cells and cellular debris. Within the main cell population, mean fluorescence intensity in FL1 can be quantified and compared between cells treated with test compounds and untreated control cells. Notes: a. Trypsin can be used to collect the adherent cells for performing this assay. b. The assay can be used to measure and compare glucose uptake levels in various cell types. c. Optional: To visualize the level of glucose uptake under fluorescence microscope, centrifuge plate at 400 x g for 5 minutes. Wash cells once with 500 µl ice-cold 1X Analysis Buffer, and replace with fresh 200 µl of 1X Analysis Buffer. Observe cells under fluorescence microscope using blue excitation fluorescence filter (excitation range 420 nm-495 nm).

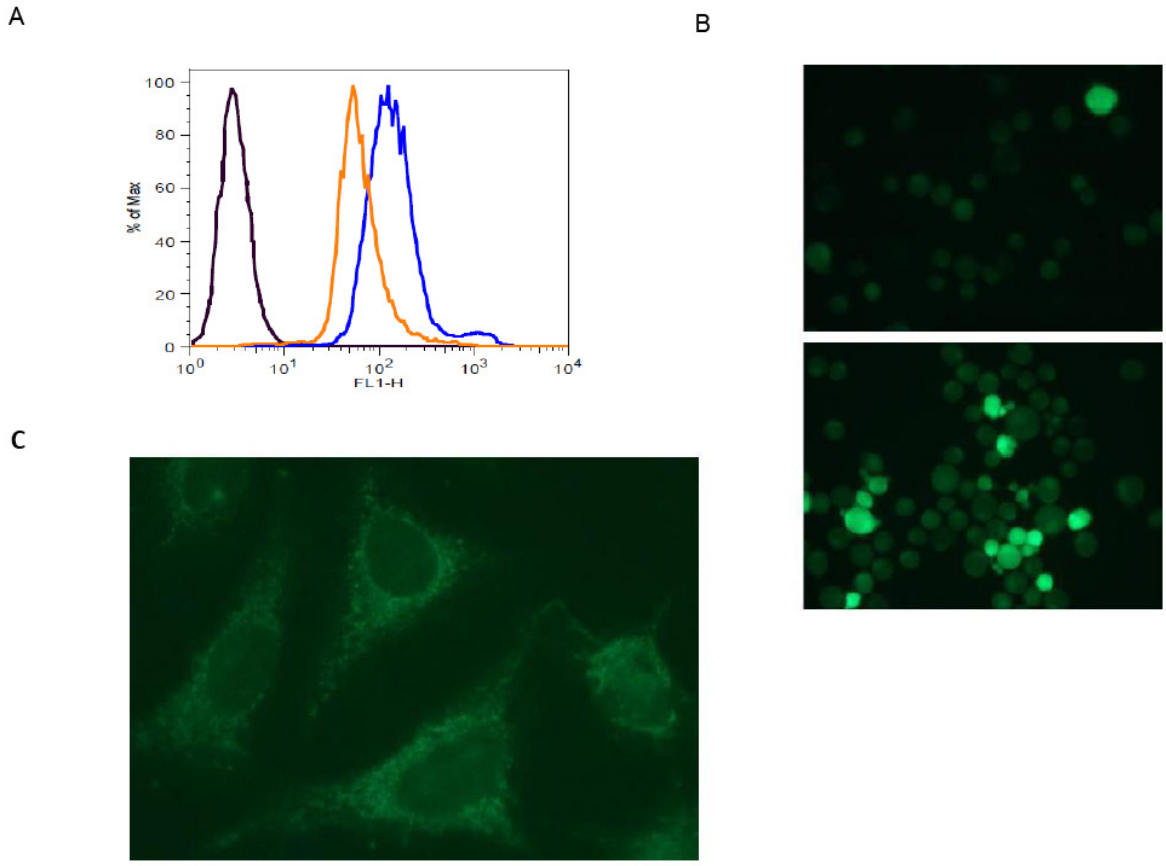

Figure: Glucose uptake in Jurkat and HeLa cells. 2.5x10^5 Jurkat cells were pretreated with or without 4 µl phloretin (1X concentration) for 45 minutes. After pretreatment, cells were washed and incubated with 2-NBDG Reagent, Glucose Uptake Enhancer, and the same concentration of phloretin for another 30 minutes according to kit's protocol. (A) Comparison of histograms from flow analysis showing the inhibition of glucose uptake by phloretin in Jurkat cells (Black: negative control cells; orange: in the presence of phloretin; blue: without phloretin). (B) Images of Jurkat cells obtained using fluorescence microscope (Top: treated with phloretin; Bottom: without phloretin treatment). (C) Glucose Uptake in HeLa cells: HeLa cells showing the uptake of 2-NBDG Reagent in the cytoplasm. Cells were stained with 2-NBDG Reagent for 30 minutes and fixed. Image was taken using a fluorescent microscope with a 60X objective lens.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

12/2017