Glucose Uptake Assay Kit (Cell-Based) (BN00905)

(Catalog # BN00905; 50 assays; Store at -20°C)

I. Introduction:

Glucose is a ubiquitous energy source in most organisms and plays a pivotal role in cellular metabolism and homeostasis. Cancer cells exhibit increased glucose uptake to support their high proliferation rate. At Assay Genie, we have developed a fluorescent glucose analog, which just like glucose can be taken up by cells through glucose transporters. However, this glucose analog 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 the 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 etc.

II. Applications:

- Measurement of glucose uptake in response to insulin, growth factors, cytokines, mitogens and nutrients, etc.
- Dual-staining of glucose transporters and glucose uptake
- Analysis of glucose metabolism and cell signaling in various cell types
- Screening of anti-diabetic compounds

III. Sample Type:

- Adherent or suspension cells

IV. Kit Contents:

Components	BN00905	Cap Code	Part Number
Analysis Buffer (50X)	1.8 ml	Brown	BN00905-1
Reagent (100X)	200 µl	Red	BN00905-2
Enhancer	1 ml	Blue	BN00905-3
Phloretin (100X)	75 µl	Yellow	BN00905-4

V. User Supplied Reagents and Equipment:

- Cell culture medium
- PBS
- 24-, 12-well tissue culture plate
- Fluorescence microscope
- Flow cytometer with excitation filter at 488 nm wavelength

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read 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.
- **GluTracker Reagent (100X):** Aliquot and store at -20^{0}C. For consistent results, avoid repeated freeze/thaw.

VII. Glucose Uptake 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 hrs, 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 hr or desired time period depending upon the test compound. To use Phloretin as a control, treat cells with 4 ul 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 hr.

   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:

Cell culture medium (0.5% FBS)	376 µl
Reagent	4 µl
Enhancer	20 µl
Test compound or 1X Phloretin	same conc. as in step 1

   Mix well. After the incubation in Step **1**, spin down the plate at 400 x g for 5 min. 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 min.

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 min. 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 with fluorescent microscope, centrifuge plate at 400 x g for 5 min. Wash cells once with 500 µl ice-cold 1X Analysis Buffer, and replace with fresh 200 µl of 1X Analysis Buffer. Observe cells under fluorescent microscope using blue excitation fluorescence filter (excitation range 420nm-495nm).

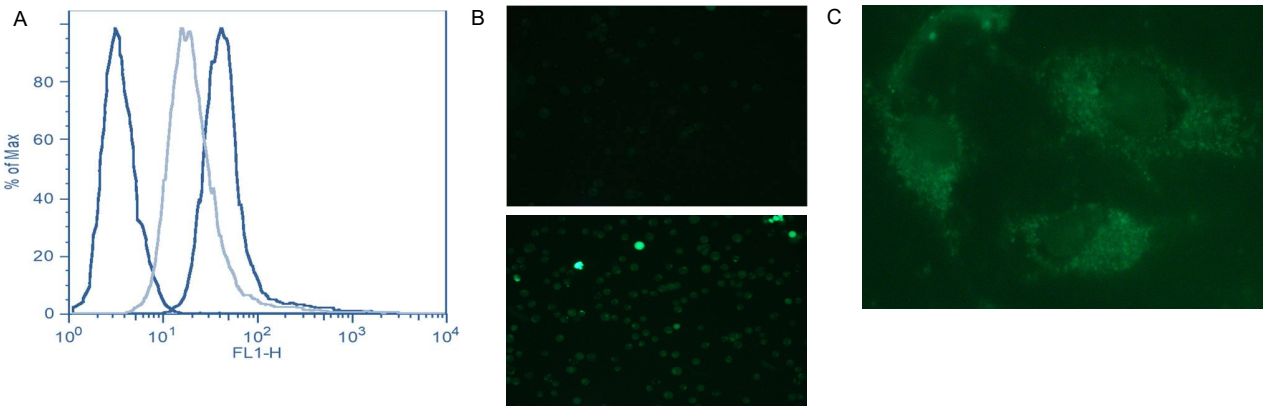

Figure: Glucose uptake in Jurkat and HeLa cells. 2.5x10^5 Jurkat cells were treated with or without 4 ul phloretin (1X concentration) for 45 min. After treatment, cells were washed and incubated with Reagent, Enhancer, and the same concentration of phloretin for another 30 min. according to the kit protocol. (A) Comparison of histogram 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 fluorescent microscope (Top: treated with phloretin; Bottom: without phloretin treatment). (C) Glucose Uptake in HeLa cells: HeLa cells showing the uptake of Reagent in the cytoplasm. Cells were stained with Reagent for 30 min. and fixed. Image was taken using a fluorescent microscope with a 60X objective lens.

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