1,5-Anhydroglucitol Uptake Assay Kit (Cell-Based) (#BN00908)

(Catalog # BN00908; 50 assays; Store at -20°C)

I. Introduction:

1,5-Anhydroglucitol (1,5-AG) is the 1-deoxy form of D-glucose. Its level in blood plasma has been used in clinical research to investigate short-term glycemic control levels in diabetic patients. Cereals and animal source proteins constitute the main 1,5-AG source for humans. Most of 1,5-AG is absorbed by renal tubules, and its levels in human body are highly regulated and relatively consistent. Glucose can act as a competitive inhibitor of 1,5-AG in blood. Therefore, glucose levels are inversely proportional to 1,5-AG concentrations. The physiological function of 1,5-AG is not well understood. Recent studies showed 1,5-AG uptake could be associated with sodium-dependent glucose transporter. Assay Genie's 1,5-Anhydroglucitol Uptake Assay Kit utilizes a proprietary fluorescent 1,5-AG analog, which can be taken up by cells. However, this 1,5-AG analog cannot be fully utilized in glycolytic processes and thus accumulates inside the cells. Fluorescence generated by this analog is proportional to the cellular 1,5-AG uptake. The assay can be used to monitor 1,5-AG uptake by using fluorescent microscopy and flow cytometry. Phloretin, a compound that can inhibit glucose transporter and 1,5-AG cell uptake, is included in the kit as a control. This non-radioactive assay kit is easy-to-use and allows qualitative and quantitative measurements of 1,5-AG uptake in cultured cells.

II. Applications:

- Measurement of 1,5-Anhydroglucitol uptake
- Elucidate cellular mechanisms of 1,5-anhydroglucitol uptake
- Screening for anti-diabetic compounds

III. Sample Type:

- Adherent or suspension cells (i.e. Jurkat, HeLa, MDA-MB-231 etc.)

IV. Kit Contents:

Components	BN00908	Cap Code	Part Number
Analysis Buffer (50X)	1.8 ml	NM	BN00908-1
Reagent (100X)	200 µl	Red	BN00908-2
Phloretin (100X) (in DMSO)	75 µl	Yellow	BN00908-3

V. User Supplied Reagents and Equipment:

- Cell culture medium
- PBS
- 24-, 12-well tissue culture plates
- 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 1 X Analysis Buffer.** Keep on ice while in use.
- **Reagent (100X):** Aliquot and store at -20°C. For consistent results, avoid repeated freeze/thaw.
- **Phloretin (100X):** Bring to room temperature. Ready to use. Store at -20°C.

VII. 1,5-Anhydroglucitol Uptake Assay Protocol:

The protocol below is for a 24-well tissue culture plate. Reagents, buffers, and cell number/well should be optimized based on cell line specifications. Assay condition optimization is strongly recommended.

1. **Sample Preparation:** Seed adherentcells (2-5x10^4 cells/well) one day before starting the assay. Adherent cells should be cultured to ~80-90% confluence. After 8-12 hrs. incubation, remove regular culture medium (supplemented with 10% FBS) and treat cells with test compound and/or vehicle control in 400 µl tissue culture medium supplemented with 0.5% FBS. Incubate cells at 37^{0}C with 5% CO_2 for 1 hr. or desired time depending upon established assay conditions. Control: treat cells with 4 µl Phloretin (final concentration 1X) in 400 µl of tissue culture medium with 0.5% FBS at 37^{0}C with 5% CO_2 for 45 min.

   Note: Cell seeding is not required for suspension cells. Seed 1-2x10^5 suspension cells/well in 400 µl of tissue culture medium with 0.5% FBS to treat with test compound or vehicle control.

2. **1,5-AG Uptake Mix:** Prepare 1,5-AG uptake mix for each well as follows:

Tissue culture medium (0.5% FBS)	up to 400 µl
Reagent (100X)	4 µl
Test compound/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 1,5-AG Uptake Mix to each well and incubate cells at 37^{0}C with 5% CO_2 for 30 min.

3. **Flow Cytometry measurement:** After incubation, for adherent cells: remove the 1,5-AG Update Mix, wash with 1X PBS, trypsinize cells, collect cells with 500 µl complete medium with (10% FBS) and keep on ice. For suspension cells, collect cells from the plate and keep on ice. Spin down at 400 x g for 5 min, remove the supernatant and wash the cell pellets twice in 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 using a 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. The, mean fluorescence intensity in FL1, within the main cell population, can be quantified and compared between untreated cells and treated cells with test compounds or using different cell types to distinguish different levels of 1,5-AG uptake.

Notes:

a. Trypsin can be used to collect the adherent cells for performing this assay.
b. The assay can be used to measure & compare 1,5-AG uptake levels in various cell types.

4. **Fluorescence Microscopy Visualization:** To visualize level of 1,5-AG uptake under fluorescence microscope, centrifuge the 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. Visualize cells under fluorescence microscope with a blue excitation fluorescence filter (excitation range 420 nm - 495 nm).

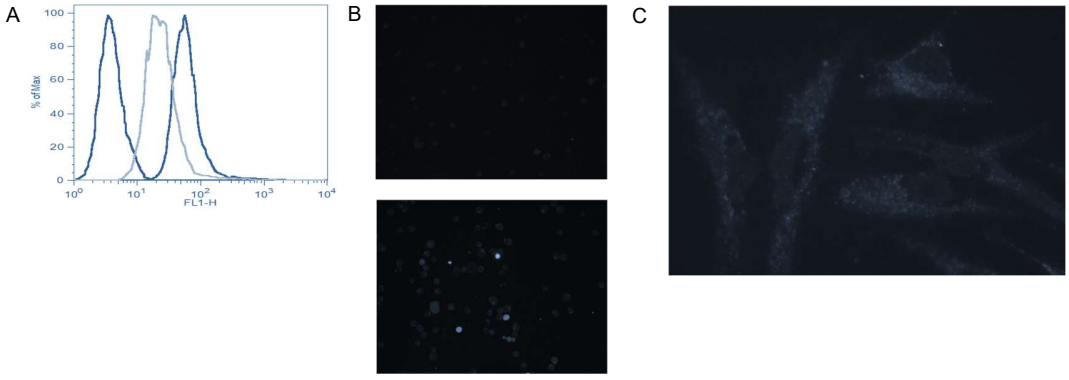

Figure: 1,5-AG uptake in Jurkat and HeLa cells. Jurkat cells ($2.5x10^5$ cells) were incubated in the presence or absence of Phloretin (4 µl, 100X concentration) for 45 min. After incubation time, cells were washed and incubated with Reagent, and the same concentration of Phloretin for another 30 min. according to kit's protocol. (A) Flow Cytometry histograms comparing the inhibition of 1,5-AG uptake by Phloretin in Jurkat cells (Black: negative control cells; orange: Phloretin-treated cells; blue: untreated cells). (B) Fluorescence microscopy images of Jurkat cells. Top: Phloretin and 1,5-AG treated cells; Bottom: 1,5-AG treated cells. (C) 1,5-AG 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 oil objective lens.

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