

ab204702

Glucose Uptake Assay Kit (Cell-based)

Instructions for Use

For measuring glucose uptake via flow cytometry and fluorescent microscopy.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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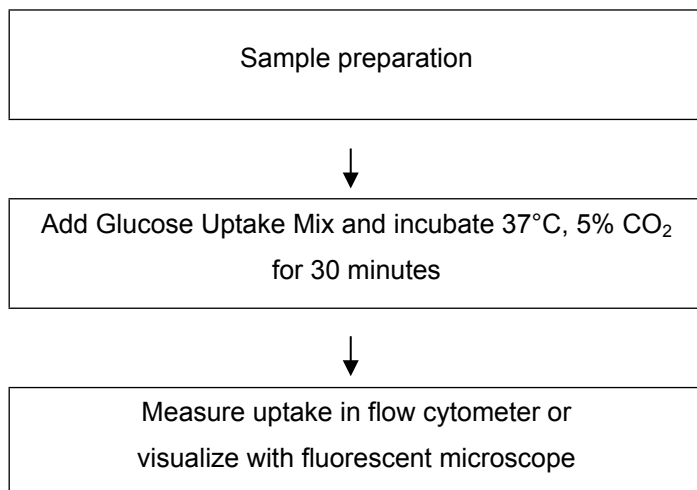
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1. BACKGROUND

Glucose Uptake Assay Kit (Cell-based) (ab204702) is an easy-to-use, non-radioactive kit allows imaging and accurate measurement of glucose uptake in cultured cells in response to insulin and growth factors. The assay is based on 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.

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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Buffer I/Analysis Buffer (50X)	1.8 mL	-20°C	-20°C
100X GluTracker Reagent/GluTracker Reagent (100X)	200 µL	-20°C	-20°C
Glucose Uptake Enhancer/GluTracker Enhancer	1 mL	-20°C	-20°C
100X Phloretin/Phloretin (100X)	75 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- CO2 incubator (37°C), tissue culture plasticware
- Cell culture medium
- Fetal Bovine Serum (FBS)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Tissue culture plates: 12-well and 24-well tissue culture plate
- Fluorescent microscope equipped with blue excitation fluorescence filter – Ex/Em = 420/495 nm.
- Standard flow cytometer equipped with a blue laser (488 nm).

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure the heat block/water bath and detection instrument are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

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9.1 **Buffer I/Analysis Buffer (50X):**

Dilute the Buffer I/Analysis Buffer (50X) with 1X PBS to make 1X Buffer I/Analysis Buffer. Store at -20°C for longer storage. Keep on ice while in use.

9.2 **100X GluTracker Reagent/GluTracker Reagent (100X):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot reagent so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw.

9.3 **Glucose Uptake Enhancer/GluTracker Enhancer:**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot enhancer so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw.

9.4 **100X Phloretin/Phloretin (100X):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot control so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. SAMPLE PREPARATION

General Sample information:

- Cells should be maintained via standard tissue culture practices. Always make sure that cells are healthy and in the log phase of growth before using them for an experiment.
- 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.
- The assay can be used to measure and compare glucose uptake levels in various cell types.
- Trypsin can be used to collect the adherent cells for performing this assay.

10.1 Cell (adherent or suspension) samples:

10.1.1 Seed adherent cells ($2 - 5 \times 10^4$ cells/well) one day before starting the assay.

10.1.2 After 8 – 12 hours, remove regular culture medium (10% FBS) and treat cells with test compound or vehicle control in 400 μ L cell culture medium with 0.5% FBS.

Suspension cells = Cell seeding is not required for suspension cells. Use up to $1-2 \times 10^5$ suspension cells/well in 400 μ L of cell culture medium with 0.5% FBS to treat with test compound or vehicle control.

10.1.3 Incubate cells at 37°C with 5% CO₂ for 1 hour (or desired time period depending upon the test compound).

Positive control: To use Phloretin as a control, treat cells with 4 μ L Phloretin (final concentration 1X) in 400 μ L of cell culture medium with 0.5% FBS at 37°C with 5% CO₂ for 1 hour.

11. ASSAY PROCEDURE

Equilibrate all materials and prepared reagents to room temperature prior to use.

- It is recommended to assay all controls and samples in duplicate.

11.1 Glucose Uptake Mix:

Prepare 400 μ L of Glucose Uptake Mix for each reaction:

Component	Glucose Uptake Mix (μ L)
Cell culture medium (0.5% FBS)	376
GluTracker Reagent	4
Glucose Uptake Enhancer/ GluTracker Enhancer	20
Test Compound (or 1X Phloretin in control)	Same conc. as in sample preparation

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Glucose Uptake Mix to ensure consistency. We recommend the following calculation: X μ L component x (Number reactions +1).

- 11.2 After the incubation with test compound (or 1X Phloretin in the positive control well), spin down the plate at 400 x g for 5 minutes. and carefully remove the medium without disturbing cells.
- 11.3 Gently add 400 μ L Glucose Uptake Mix to each well and incubate cells at 37°C with 5% CO₂ for 30 minutes.
- 11.4 After incubation, collect cells from the plate and keep on ice.

12.FLOW CYTOMETRY DETECTION

- **The cellular green fluorescence signal should be measured using a flow cytometer with identical equipment settings in all tubes.**
 - 12.1 After incubation (Step 11.4), collect cells from the plate and keep on ice.
 - 12.2 Wash once with 1 mL ice-cold 1X Buffer I/Analysis Buffer.
 - 12.3 Spin down at 400 x g for 5 minutes
 - 12.4 Resuspend cell pellet in 400 μ L ice-cold 1X Buffer I/Analysis Buffer.
 - 12.5 Cells are ready to be analyzed.
 - 12.6 Within the flow cytometry software, set an FSC-SSC dot plot and an FL1 (488 nm excitation laser) histogram plot. For better separation of the different cell populations, using a log scale is recommended for the fluorescence channel FL1.
 - 12.7 Run unstained cells and adjust both forward and side scatter PMT amplifications to display all the cell subsets on the FSC-SSC plot.
 - 12.8 Select the main cell population in the FSC vs SSC plot to exclude dead cells and cellular debris.
 - 12.9 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.

13.FLUORESCENCE MICROSCOPY DETECTION

- 13.1 After incubation (Step 11.4), centrifuge plate at 400 x g for 5 minutes.
- 13.2 Wash cells with 500 μ L ice-cold 1X Buffer I/Analysis Buffer.
- 13.3 Replace with fresh 200 μ L of 1X Buffer I/Analysis Buffer.
- 13.4 Observe cells under fluorescent microscope using blue excitation fluorescence filter (excitation range 420nm-495nm).

14. TYPICAL DATA

Glucose uptake in Jurkat and HeLa cells. 2.5×10^5 Jurkat cells were treated with or without 4 μL phloretin (1X concentration) for 45 minutes. After treatment, cells were washed and incubated with GluTracker Reagent, Glucose Uptake Enhancer/GluTracker Enhancer, and the same concentration of phloretin for another 30 minutes according to the kit protocol.

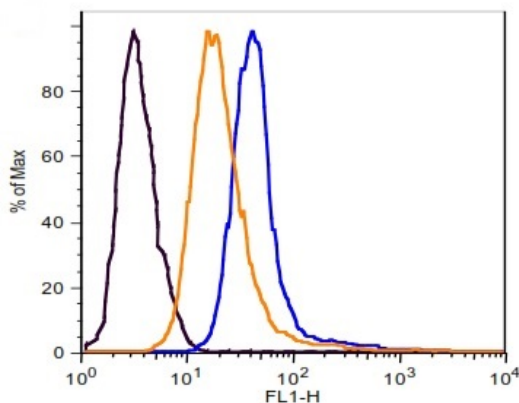


Figure 1. Comparison of histogram from flow analysis showing the inhibition of glucose uptake by phloretin in Jurkat cells (Black: negative control cells; orange: in the presence of phloretin; blue: without phloretin).

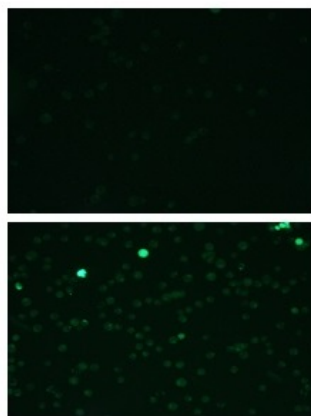


Figure 2. Images of Jurkat cells obtained using fluorescent microscope (Top: treated with phloretin; Bottom: without phloretin treatment).

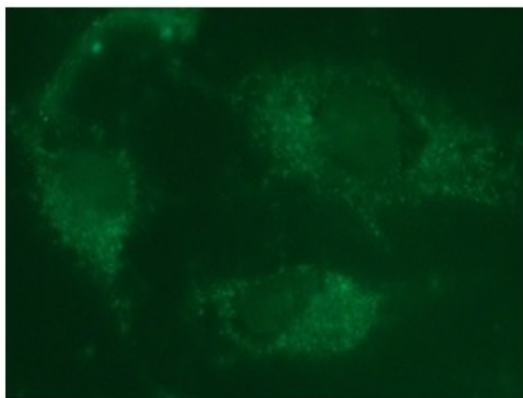


Figure 3. Glucose Uptake in HeLa cells: HeLa cells showing the uptake of GluTracker Reagent in the cytoplasm. Cells were stained with GluTracker Reagent for 30 minutes, and fixed. Image was taken using a fluorescent microscope with a 60X objective lens.

15. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Seed adherent cells ($2-5 \times 10^4$ cells/well) one day before starting the assay
- Remove regular culture medium (10% FBS) and treat cells with test compound or vehicle control with cell culture medium (0.5% FBS)
- Incubate cells at 37°C with 5% CO₂ for 1 hour or desired time period depending upon the test compound.
- Prepare Glucose Uptake Mix (Number samples +1).

Component	Glucose Uptake Mix (μL)
Cell culture medium (0.5% FBS)	376
GluTracker Reagent	4
Glucose Uptake Enhancer/GluTracker Enhancer	20
Test Compound (or 1X Phloretin in control)	Same conc. as in sample preparation

- Add 400 μL of Glucose Uptake Mix to the sample wells.
- Incubate plate at 37°C 5% CO₂ 30 minutes protected from light.
- Analyze cells using a flow cytometer or with fluorescent microscope.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
Lower/ Higher readings in samples	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting

17. FAQ

18. NOTES

Technical Support

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