Novel Method for the Detection of Glucose Uptake: Direct Measurement of Glucose Levels in Cultured Cells

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Abstract

Glucose uptake is the transport process of glucose from the outside of cells into cells across the cell membrane. It is one of the key regulatory steps of glucose metabolism. Study of glucose uptake provides important information for understanding glucose metabolism and its regulation in normal and disease development. Here we report the development of a novel glucose uptake detection method based on direct measurement of glucose levels in cultured cells. In this method, we utilized the inhibitor, 3-bromopyruvate (3BP), to temporarily inhibit hexokinases, the first enzyme that metabolizes glucose in cells. Thus, glucose metabolism was blocked, which leads to glucose accumulation rather than its rapid metabolism inside the cells. Glucose uptake was then detected by direct measurement of the glucose levels inside of the cells using Glucose Oxidase/HRP/OxiRed™ enzyme based method. In comparison with current widely used methods, this direct glucose uptake detection method has the following advantages: i) No radioisotope materials are used; ii) Glucose uptake directly detected glucose without using structurally modified glucose derivatives; iii) Glucose from culture medium is transported inside of cells, and then detected with minimum culture condition changes and washing steps. This simple, sensitive and direct glucose uptake detection method provides a powerful tool for studying the process of glucose uptake and its regulation, as well as for screening and characterization of drugs that regulate glucose uptake during normal and disease development.

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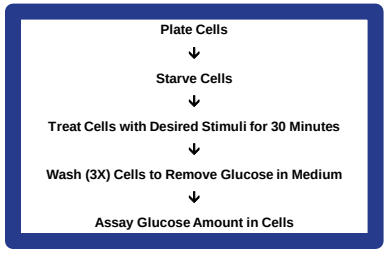
Experimental Flow Chart



3BP Inhibits Hexokinase Enzyme Activity

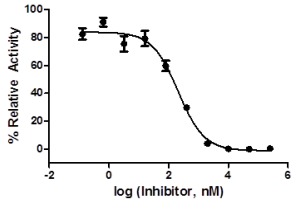


Figure 1. Different doses of 3PB were added into purified Human Hexokinase enzyme solution. The Hexokinase activity was measure using Hexokinase Colorimetric Assay Kit (BioVision Cat# K789-100) following the kit protocols.

Detection of Glucose Uptake In the Presence of 3BP in Jurkat Cells Stimulated by Serum

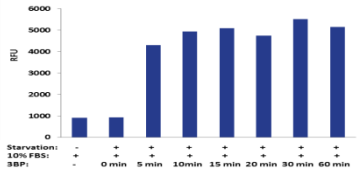


Figure 2: Jurkat cells were serum starved for 5 hours in culture medium (with glucose). 0.5 mM 3BP and 10% Fetal Bovine Serum (FBS) was then added into the culture, and incubated for different times. Glucose level were measured following the standard procedure using Glucose Assay Kit (BioVision Inc. Cat# K606-100).

Detection Of Glucose Uptake of 3T3-L1 Cells Stimulated by Insulin

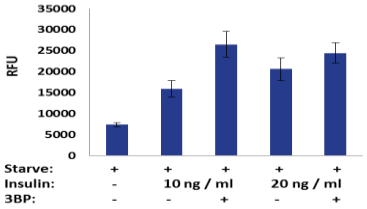


Figure 3: 3T3-L1 cells were plated over night, then serum starved for 24 hours. 0.5 mM 3BP and 10 or 20 ng/ml Insulin were then added to stimulate glucose uptake for 30 min. Glucose in cells were detected using the standard procedure described above.

Glucose Transporter Inhibitor Inhibits Insulin Stimulated Glucose Uptake in 3T3-L1 Cells

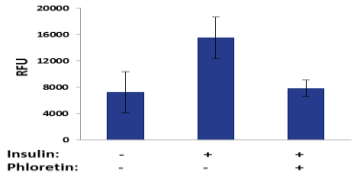


Figure 4: Differentiated 3T3-L1 cells were starved for 24 hours and treated with Insulin (20ng/ml) and Phloretin ( ) for 30 min. Glucose levels were measured following the standard procedure described above.

Discussions

1. **In the absence of 3BP, glucose is rapidly metabolized by cytosolic enzymes during sample preparation and assay processes, and therefore glucose uptake in cells cannot be accurately detected (data not shown). In this report, we used a widely utilized hexokinase inhibitor, 3-Bromopyruvate (3BP), to inhibit hexokinase activities to block glucose metabolism in cells, therefore glucose uptake can be measured accurately.**

2. **Although a few other glucose uptake assay methods have been developed and commercially available, such as, Isotope labeled glucose, florescent dye labeled glucose, or 2-deoxy-D-glucose (2DG) based methods. The isotope method involved tedious isotope labelling, and associated with expensive isotope and waste costs and biohazard. Other methods involved in using structurally altered glucose derivatives, so it is questionable whether the structurally altered glucose derivatives truly represent native glucose behaviors during glucose uptake processes. In addition, glucose in normal culture medium has to be removed, which created another alteration of cell culture condition that may alter glucose uptake behaviors of cells. In the method we developed here, glucose in culture medium are directly used, without glucose structural alteration, so it represents true glucose behaviors for study of glucose uptake in cells.**

3. **3BP is used to block glucose metabolism in the new method, although it is used for only short time (30 min), but we currently have not ruled out whether 3BP affect other cellular functions so altering glucose uptake behavior of cells.**

Applications

1. **Study the basic mechanism and regulation of glucose uptake during normal and disease development processes.**

2. **Study cell signaling of growth factors, cytokines or other regulatory factors that regulate glucose uptake in various cell types.**

3. **Screen, and characterize drugs that stimulate or inhibit glucose uptake.**

Advantages

1. **Use and detect native glucose directly, no glucose structural alteration, no fluorescence dye labelling, no isotope.**

2. **No need of removing glucose from cell culture condition.**

3. **Simple, convenient, easy to adapt to high throughput (HT) assay for screening large number of samples**

Conclusions

A novel glucose uptake detection method is developed. The new method provides a very useful tool for the study of glucose uptake, metabolic diseases, as well as study of cell signaling that regulate glucose uptakes, and screening drugs that stimulate or inhibit glucose uptake.

The method has been developed and commercialized as a product. For more information, visit www.biovision.com Direct Glucose Uptake Assay Kit, Cat# K924-100.

Related Products

Glucose Uptake Colorimetric Assay Kit (K676)
GluTrackerTM Glucose Uptake Assay Kit (K681)
Glucose Assay Kit (K606)
Lactate Assay Kit (K627)
Glucose Uptake Fluorometric Assay Kit (K666)
1, 5-Anfydroglucitol Uptake Assay Kit (K684)
Glycogen Assay Kit (K646)
ATP Colorimetric/Fluorometric Assay Kit (K354)