A Non-Radioactive Photometric Assay for Glucose Uptake in Insulin-Responsive 3T3-L1 Adipocytes

Qin Zhao, Jinfang Liao and Zhenjun Diwu
AAT Bioques, Inc., 520 Mercury Drive, Sunnyvale, CA 94085

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Introduction

Glucose transport systems are responsible for transporting glucose across cell surface membranes. The rate of glucose uptake in cells is tightly regulated by factors including insulin. Measuring uptake of glucose has been dominated by traditional C^{14} labeled 2-deoxyglucose (2-DG) radioactive measurement. However, the routine use of a radio-labeled probe is costly, requires a special handling procedure, and the results must be corrected by separating the extracellular signal.

We have developed an enzymatic photometric assay detecting for 2-DG uptake. The entire process from cell culture to glucose uptake evaluation could be completed in the single well of a 96-well microplate.

Assay Principle

2-DG were taken up by adipocytes through insulin stimulation, and metabolized to a non-metabolizable metabolite, 2-Deoxy Glucose-6-Phosphate (2-DG6P). Cells were lysed, and the amount of the accumulated 2-DG6P was proportional to the glucose uptake by cells. 2-DG6P in cell lysate was enzymatically coupled to the generation of NADPH, which is specifically monitored by a NADPH sensor.

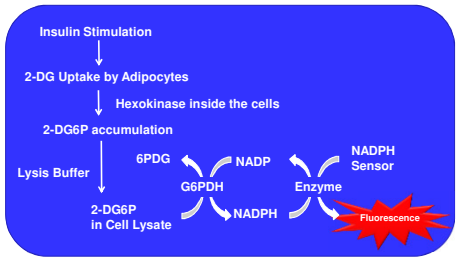
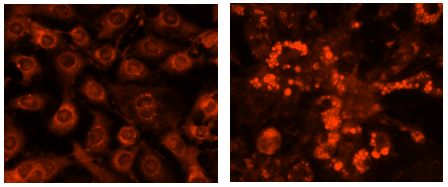


Figure 1. Assay principle of insulin stimulated glucose uptake measurement via fluorescence detection

Preparation of 3T3-L1 Adipocytes

1. 3T3-L1 fibroblasts were grown 2 days post-confluence in a 75 cm flask with DMEM supplemented with 10% FBS.
2. Differentiation: The cells were incubated 2 days with DMEM supplemented with 10% FBS, 0.83µM insulin, 0.25µM dexamethasone, and 0.25mM isobutylmethylxanthine.
3. Maintenance: The cells were then maintained for 2 days with DMEM supplemented with 10% FBS and 0.83µM insulin alone.
4. The medium was changed to DMEM supplemented with 10% FBS for another 3-5 days.
5. Differentiated cells (at least 95% of which showed an adipocyte phenotype by accumulation of lipid droplets) were used on day 8 to 12 after induction of differentiation.



3T3-L1 Pre-adipocytes **3T3-L1 Adipocytes**

Figure 2. 3T3-L1 cell Images before and after differentiation, lipid droplets are stained with Nile Red.

Assay Protocol

Plate cells 50,000-80,000 cells/well in a 96-well plate	
Incubate with FBS free medium	6-16 hours
Insulin (1µM) Stimulation	20 min
2-DG (1mM) Uptake	40 min
Add lysis buffer to lyse cells	20 min
Neutralization cell lysate	5 min
Add assay mixture	30-60 min

Entire Assay was Completed in a 96-Well Plate Format in One Day!

Assay Results by Fluorescence

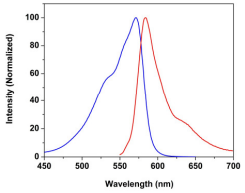
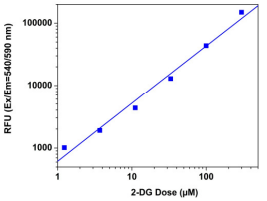


Figure 3A. The excitation and fluorescence spectra of the assay mixture with 100 µM 2-DG added in the assay system.

Figure 3B. 2-DG dose response was measured in a 96-well plate with fluorescent NADPH sensor at Ex/Em =540/590 nm. As low as 3 µM 2-DG can be detected with 30 minutes incubation.



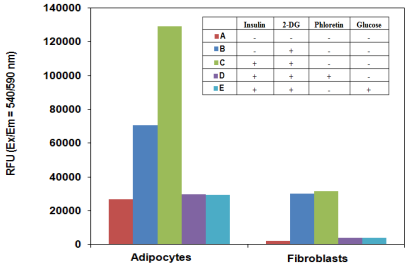


Figure 3C. Measurement of 2-DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed with in a 96-well black wall/clear bottom cell culture Poly-D lysine plate using a Flexstation microplate reader (Molecular Devices).

Assay Results by Absorbance

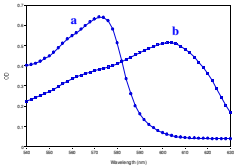
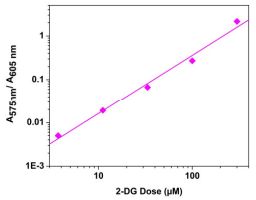


Figure 4A. Absorption spectra of the assay mixture with a: 100 µM 2-DG in the assay system, b: 0 µM 2-DG in the assay system.

Figure 4B. 2-DG dose response was measured in a 96-well plate with NADPH sensor at absorbance ratio of 575 nm to 605 nm. As low as 10 µM 2-DG can be detected with 30 minutes incubation.



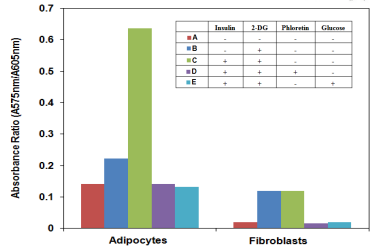


Figure 4C. Measurement of 2-DG uptake in differentiated 3T3-L1 adipocytes and 3T3-L1 fibroblasts. Assays were performed in a 96-well black wall/clear bottom cell culture Poly-D lysine plate using a SpectraMax microplate reader (Molecular Devices).

Summary

- This non-radioactive enzymatic assay only takes 2~3 hours in a 96-well microplate format.
- It is very efficient and safe without radioactivity involved.
- It can be used for the high throughput screening of glucose uptake regulators and inhibitors.