A Bioluminescent Assay Enables Easy Measurement of Glucose Uptake

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Abstract # 2107

1. Introduction

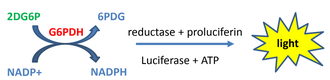
Glucose uptake is an important pharmacological target. Cancer cells support their high rates of proliferation by overexpressing glucose transporters to increase their rates of glucose uptake. In contrast, the decreased rates of glucose uptake in diabetic fat and muscle cells can lead to hyperglycemia and its inherent deleterious biological effects. Hence, effectors of glucose uptake would be useful for both anticancer therapies and diabetes management. The standard method for measuring glucose uptake has long been the addition of a radioactive glucose analog (2-deoxyglucose) and measurement of the accumulation of the stable and impermeable phosphorylated derivative, 2-deoxyglucose-6-phosphate (2DG6P). In the interest of transitioning to a safer, non-radioactive assay, we have developed a simple bioluminescent glucose uptake assay that measures the production of NADPH through the oxidation of 2DG6P by glucose-6-phosphate dehydrogenase (G6PDH). This assay is both rapid and convenient and exhibits a larger signal window than comparable fluorescent or colorimetric approaches. One can use the assay in 96- and 384-well formats, apply it to various sample types, and multiplex it with other assays (such as cell viability or cytotoxicity) to maximize data per well.

2. Assay Concept and Protocol

- Add 2-deoxyglucose (2DG) to cells
  - 2DG is uptaken and converted to 2-deoxyglucose-6-phosphate (2DG6P)

- Add Stop/Go Buffers
  - Ends uptake, lyses cells, and inactivates proteins

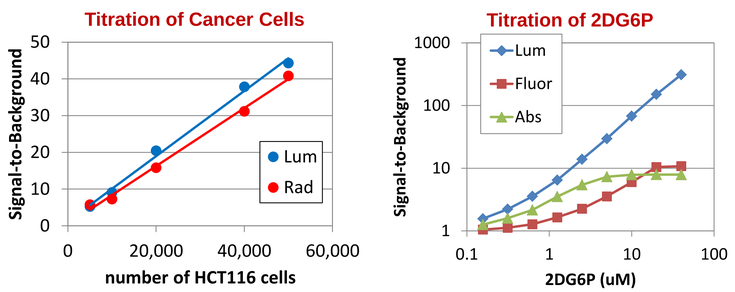
- Add 2DG6P Detection Reagent
  - Couples 2DG6P to production of light through glucose-6-phosphate dehydrogenase (G6PDH)



Treat cells as desired (96-well plate)

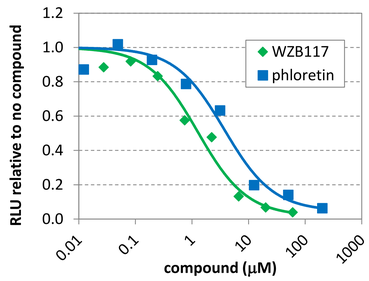
- Remove media
- (If glucose present, wash 1x w/ 100 ul PBS)
- Add 50 ul 1 mM 2DG
  - ↓ incubate 10 min
- Add 25 ul Stop Buffer
  - ↓ shake briefly
- Add 25 ul Go Buffer
  - ↓ shake briefly
- Add 100 ul 2DG6P Detection Reagent
  - ↓ incubate 60 min
- Read luminescence

3. Assay Comparison



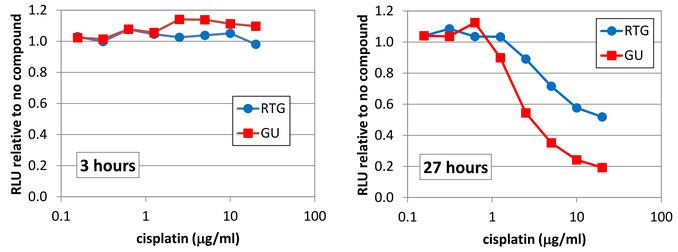
Glucose uptake by HCT116 cells initiated with 1mM 2DG for 10 minutes was measured with the luminescent and radioactive assays (left graph). A titration of 2DG6P was assayed by luminescent, fluorescent, and absorbance methods (right graph).

4. Measurement of Glucose Transporter Inhibition



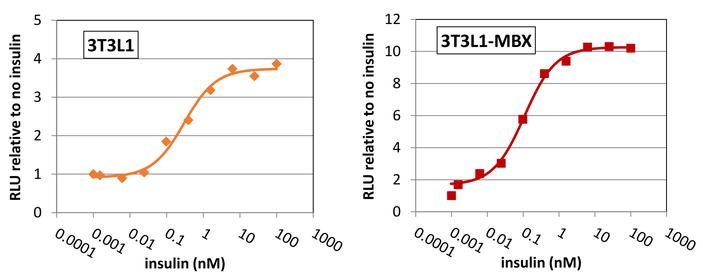
Ten thousand (10,000) HCT116 cells were treated with a titration of glucose transporter inhibitors in a volume of 50μl for 10 minutes. Uptake was initiated with 5 μl of 10mM 2DG for 10 minutes before addition of Stop Buffer.

5. Multiplexing Viability and Glucose Uptake



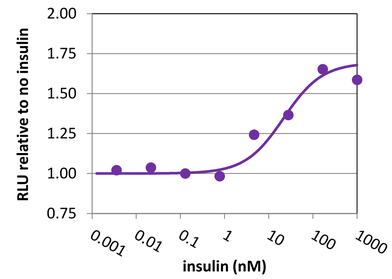
Ten thousand (10,000) OVCAR-3 cells were treated with media containing 1x RealTime-Glo (RTG) and a titration of cisplatin for 3 and 27 hours. The luminescent signal from RTG was read immediately before the glucose uptake assay (GU).

6. Insulin Stimulation of Adipocytes



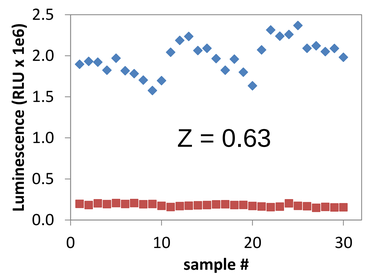
Adipocytes were differentiated in a 96-well plate and treated with a titration of insulin for 1 hour. Uptake was initiated with 1mM 2DG for 10 minutes before addition of Stop Buffer.

7. Insulin Stimulation of Myotubes



L6 myotubes were differentiated in a 96-well plate and treated with a titration of insulin for 1 hour. Uptake was initiated with 0.1mM 2DG for 30 minutes before addition of Stop Buffer.

8. Z-factor in 96-well plate



Sixty wells of a 96-well plate were seeded with 20,000 HCT116 cells per well. After treatment with 25μl PBS +/- 50μM cytochalasin B (30 wells for each condition) for 5 minutes, 25μl PBS + 2mM 2DG were added for 10 minutes before addition of Stop Buffer.

9. Conclusions

The bioluminescent glucose uptake assay

- Is simple & sensitive
- Produces results equivalent to the radioactive assay
- Can be multiplexed with other assays to get more information per well

Suitable to detect

- Inhibitors of glucose uptake
- The insulin response of insulin sensitive cells
- Changes in glucose uptake in response to changes in metabolism

For interest in these assay reagents, please contact the corresponding author.

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