A Bioluminescent Assay Enables Easy Measurement of Glucose Uptake

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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 G6PDH

3. Assay Comparison

4. Measurement of Glucose Transporter Inhibition

5. Multiplexing Viability and Glucose Uptake

6. Insulin Stimulation of Adipocytes

7. Insulin Stimulation of Myotubes

8. Z-factor in 96-well plate

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.