Measuring Glucose Uptake with the Glucose Uptake-Glo™ Assay and the GloMax® Discover System

Promega Corporation

Materials Required

- Glucose Uptake-Glo™ Assay (Cat.# J1341, J1342, J1343)
- GloMax® Discover System (Cat.# GM3000)
- white, 96-well assay plates (Corning Cat.# 3903)
- Phosphate-buffered saline (PBS)

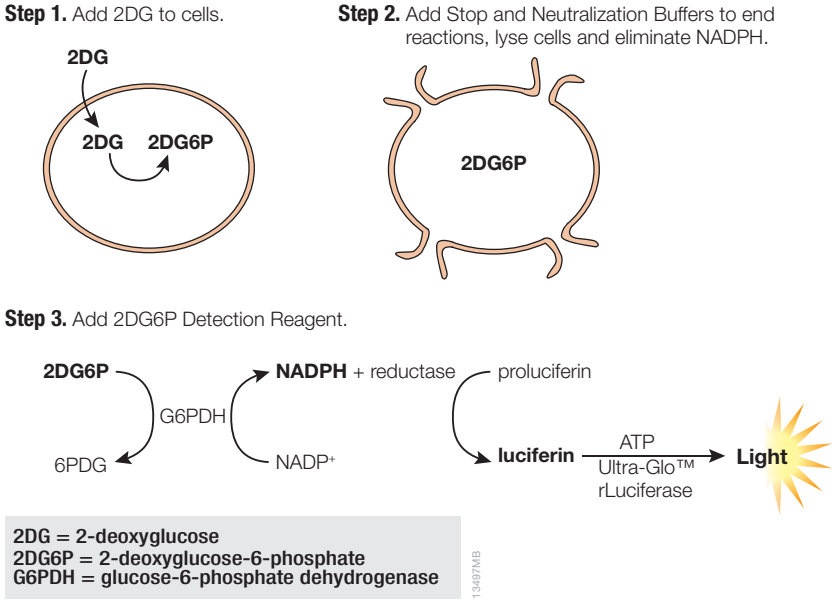
Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: The *GloMax® Discover System Operating Manual* #TM397 and *Glucose Uptake-Glo™ Assay Technical Manual* #TM457 are available at: **www.promega.com/protocols/**

Glucose is the primary source of energy for most, if not all, organisms. In general, changes in glucose uptake can reflect overall changes in metabolism. In cancer cells, glucose uptake assays can be used to monitor the over-expression of glucose transporters or to identify glucose transporter inhibitors. In fat and muscle cells, changes in GLUT4 translocation upon insulin stimulation can be observed by measuring glucose uptake. Moreover, with immunologically relevant cells, glucose uptake can also be used to follow the transformation of certain cell types from one stage to another.

The Glucose Uptake-Glo™ Assay provides a non-radioactive, plate-based, homogeneous bioluminescent method for measuring glucose uptake in mammalian cells based on the detection of 2-deoxyglucose-6-phosphate (2DG6P). When 2-deoxyglucose (2DG) is added to cells, it is transported across the membrane and rapidly phosphorylated in the same manner as glucose. However, enzymes that further modify glucose-6-phosphate (G6P) cannot modify 2DG6P, and thus this membrane-impermeable analyte accumulates in the cell. After a brief incubation period, an acid detergent solution (Stop Buffer) is added to lyse the cells, terminate uptake and destroy any NADPH. A high-pH buffer solution (Neutralization Buffer) is then added to neutralize the acid, and Detection Reagent (glucose-6-phosphate dehydrogenase (G6PDH), NADP+, reductase, luciferase and a proluciferin substrate) is added to the sample wells. G6PDH oxidizes 2DG6P to 6-phosphodeoxygluconate and simultaneously reduces NADP+ to NADPH. The reductase uses NADPH to convert the proluciferin to luciferin, which is then used by luciferase to produce a luminescent signal that is proportional to the concentration of 2DG6P.

This Application Note provides a protocol for measuring glucose uptake using the Glucose Uptake-Glo™ Assay and the GloMax® Discover System. Measuring the luminescence from the Glucose Uptake-Glo™ Assay is easy on the GloMax® Discover because the protocol comes preloaded on the instrument. In addition, the extended dynamic range and minimal well-to-well cross talk of the GloMax® Discover System allow you to easily measure signals of varying intensities on the same plate.



Glucose Uptake-Glo™ Assay Protocol

For detailed instructions and assay notes for various volumes and plate formats, see the *Glucose Uptake-Glo™ Assay Technical Manual* #TM467. The following protocol is performed in 96-well plates.

1. Treat cells as desired. Remove medium and wash with 100µl PBS if glucose is present.

   Note: To most efficiently remove glucose from cell cultures, remove culture media and PBS slowly using a pipettor

2. Add 50µl of prepared 1mM 2DG per well, shake briefly, and incubate for 10 minutes at room temperature. Optimal cell number and incubation time will vary for different cell types (See Section 3.B of the *Glucose Uptake-Glo™ Assay Technical Manual*, TM467, for details). If the medium does not contain glucose, a concentrated aliquot (above 1mM) of 2DG can be added directly to the cells without removing medium (e.g., add 5µl of 10mM 2DG to a 50µl sample).
3. Add 25µl of Stop Buffer. Shake briefly.
4. Add 25µl of Neutralization Buffer and shake briefly.
5. Add 100µl of 2DG6P Detection Reagent and shake briefly. If fewer dispensing steps are desired, the Neutralization Buffer may be added to the 2DG6P Detection Reagent just prior to assay, and the combination can be added in a volume of 125µl.

   Note: Be sure to prepare 2DG6P Detection Reagent 1 hour before use to minimize assay background
6. Incubate 0.5–5 hours at room temperature.
7. Measure luminescence using a 0.3–1 second integration on the GloMax® Discover by selecting the Glucose Uptake-Glo™ protocol.

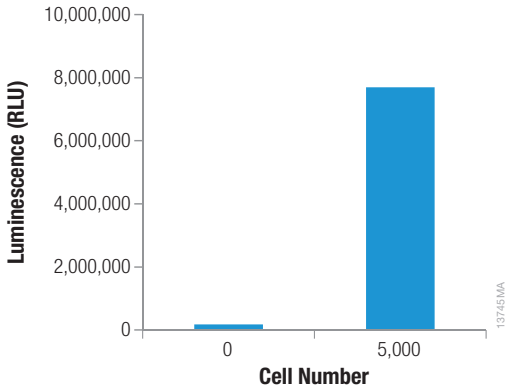


Figure 1. Glucose Uptake-Glo™ applied to HCT116 colon cancer cells. In a volume of 100µl, 0 (control) or 5,000 cells were assayed 24 hours after plating in a 96-well plate. The signal from 5,000 cells was 42-fold higher than that of the no-cell background control.

Conclusion

The GloMax® Discover can detect luminescence generated using the Glucose Uptake-Glo™ Assay (Figure 1). The uptake time is dependent on the number and type of cells being studied, but the assay can detect glucose uptake from as few as 5,000 cells. This detection sensitivity together with a threefold signal-to-background ratio make Glucose Uptake-Glo™ one of the simplest multiwell plate assay methods available.

The GloMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range, and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. GloMax® Discover allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made easy with a variety of options, including data export to your local network.

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