

Enhanced glucose uptake under hypoxic conditions in MCF7 breast cancer and U-2 OS osteosarcoma cells

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Introduction

In most solid tumors, hypoxia drives reliance on glucose uptake and glycolysis. Robust, non-radioactive methods to quantify glucose uptake under controlled oxygen conditions are essential for studying cancer metabolism and evaluating metabolic inhibitors. Here, we assessed hypoxia-induced changes in glucose uptake and viability in MCF7 and U-2 OS cancer cell lines using a luminescent assay workflow on the SpectraMax® iD5e Multi-Mode Microplate Reader with SpectraMax® aer Gas Mixer.

Hypoxic culture conditions resulted in a significant increase in glucose uptake compared to normoxic controls, consistent with hypoxia-driven metabolic adaptation. Inhibition of glucose transport led to a marked reduction in measured glucose uptake, demonstrating assay sensitivity and pathway relevance. Cell viability showed a significant reduction under hypoxic conditions.

These results demonstrate a reproducible workflow for measuring glucose uptake and cell viability under physiologically relevant oxygen conditions. The combination of controlled hypoxia with sensitive luminescent detection provides a powerful platform for studying cancer metabolism and screening metabolic inhibitors.

Methods

Glucose uptake under normoxia vs. hypoxia

- MCF7 breast cancer or U-2 OS osteosarcoma cells were seeded at 20,000 cells per well in 96-well white/clear microplates and allowed to attach and grow overnight in a 37°C, 5% CO₂ incubator.
- One of two plates was kept in a 37°C, 5% CO₂ incubator, and the other was placed in the SpectraMax iD5e reader at 37°C, with gas mixer set to deliver 5% CO₂ and 1% oxygen.
- After 24 hours, a subset of cells was treated with inhibitors of glucose transporter 1 (GLUT1), phloretin and NV-5440, for 60 minutes.
- Following GLUT1 inhibitor treatment, glucose uptake was assessed using the Glucose Uptake-Glo™ Assay (Promega), with luminescent readout measured on the SpectraMax iD5e reader.

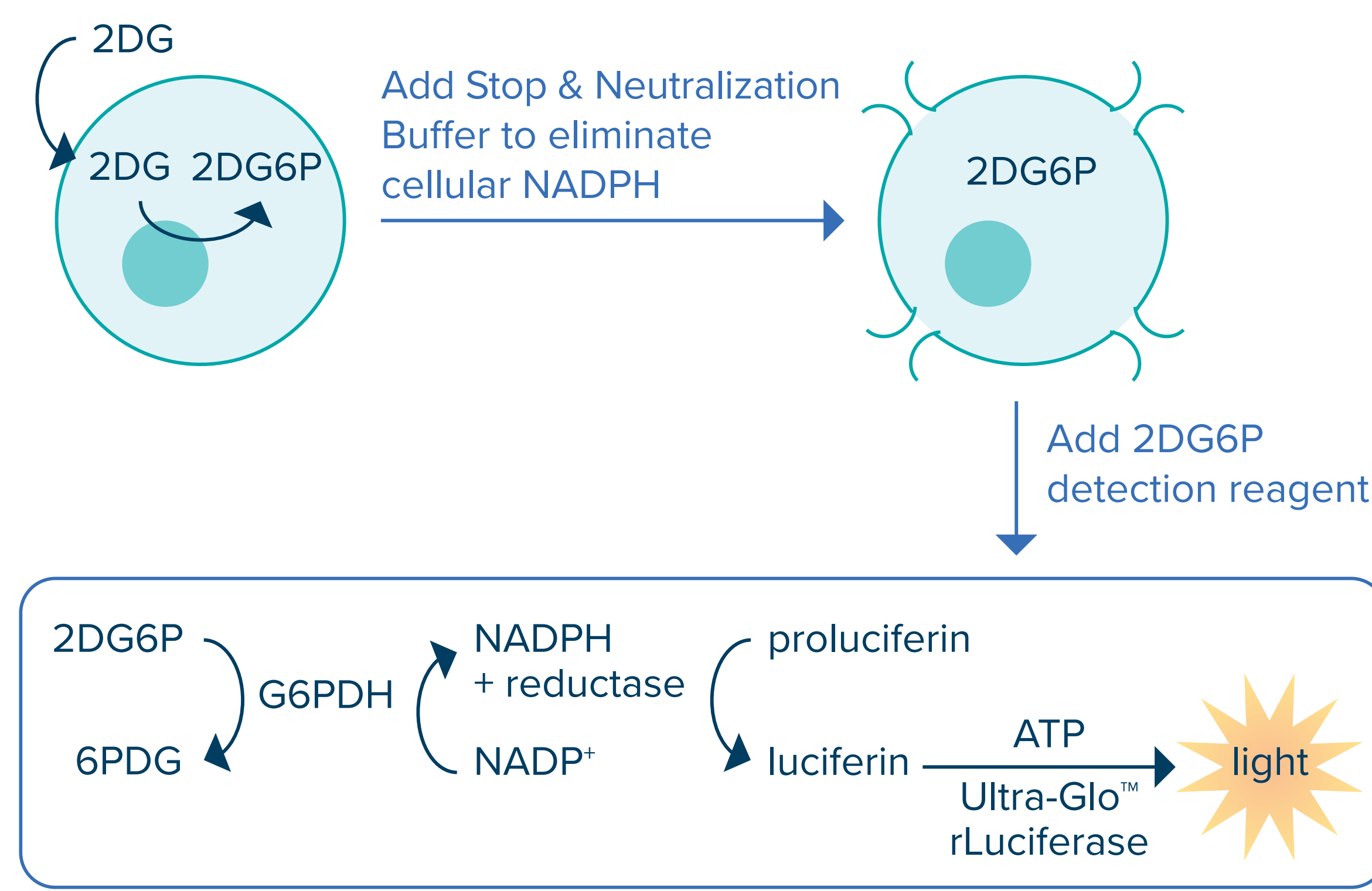


Figure 1. The Glucose Uptake-Glo assay enables detection of cellular glucose uptake as luminescence that is measured using a microplate reader.

Cell viability under normoxic & hypoxic culture conditions

- MCF7 breast cancer or U-2 OS osteosarcoma cells were seeded and cultured overnight as described above.
- Each microplate was placed in the SpectraMax iD5e Multi-Mode Microplate Reader with SpectraMax aer Gas Mixer (Molecular Devices). For the normoxic culture condition, the system was set at 37°C, with 5% CO₂ and atmospheric oxygen. For the hypoxic condition, it was set at 37°C, with 5% CO₂ and 1% oxygen.
- The RealTime-Glo™ MT Cell Viability Assay (Promega) was used to assess viability under normoxic and hypoxic culture conditions. Reagents were added to cells prior to placing them in the microplate reader. For each condition tested, cells remained in the reader for 24 hours, and viability was assessed by taking luminescence measurements every 10 minutes for 24 hours.

Results

Hypoxia measurably increases glucose uptake and decreases viability

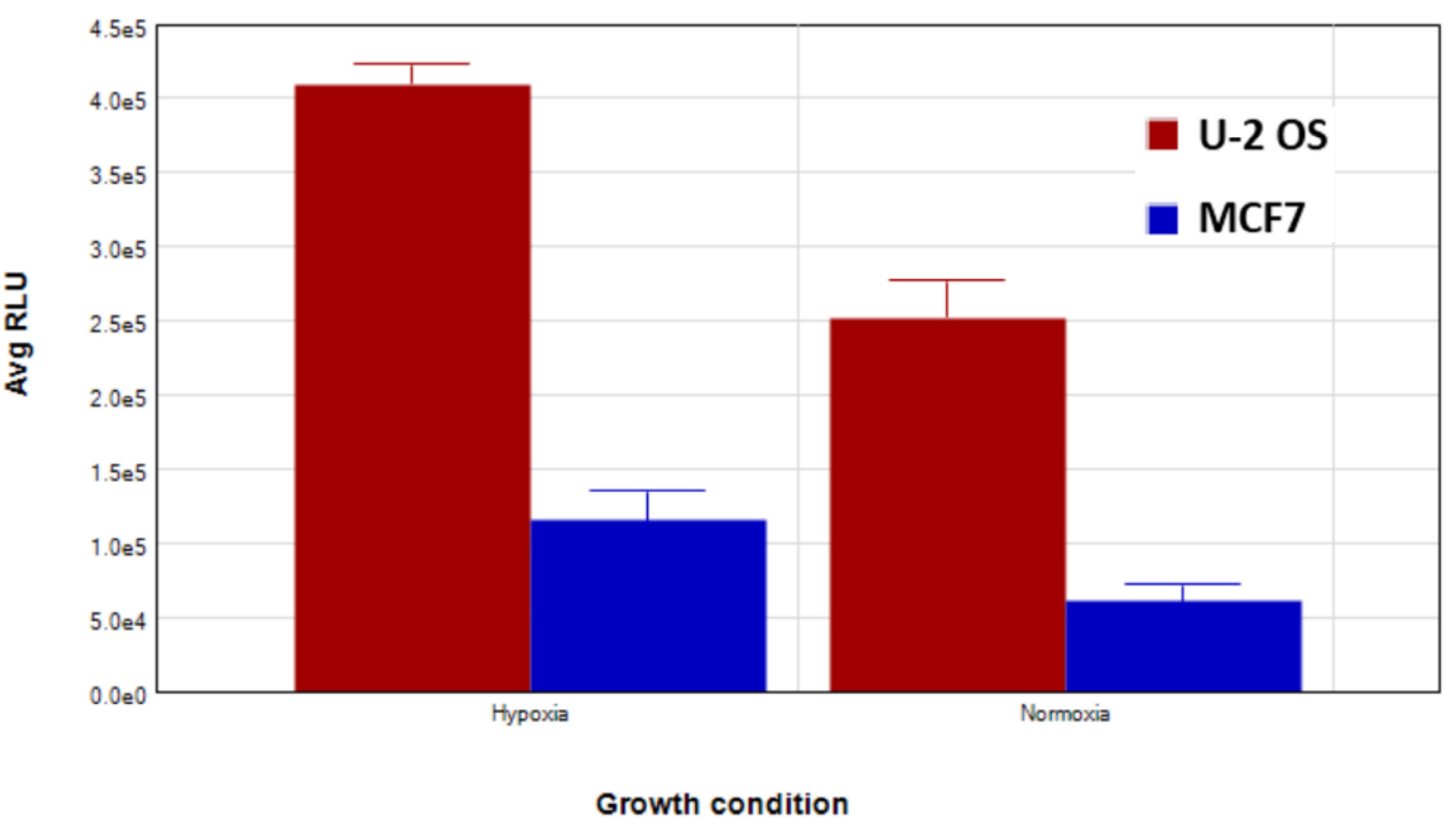


Figure 2. Glucose uptake by U-2 OS osteosarcoma and MCF7 breast cancer cell lines under hypoxic vs. normoxic conditions. Cultures were maintained under each condition using the SpectraMax aer gas mixer, and luminescence was detected with the SpectraMax iD5e microplate reader.

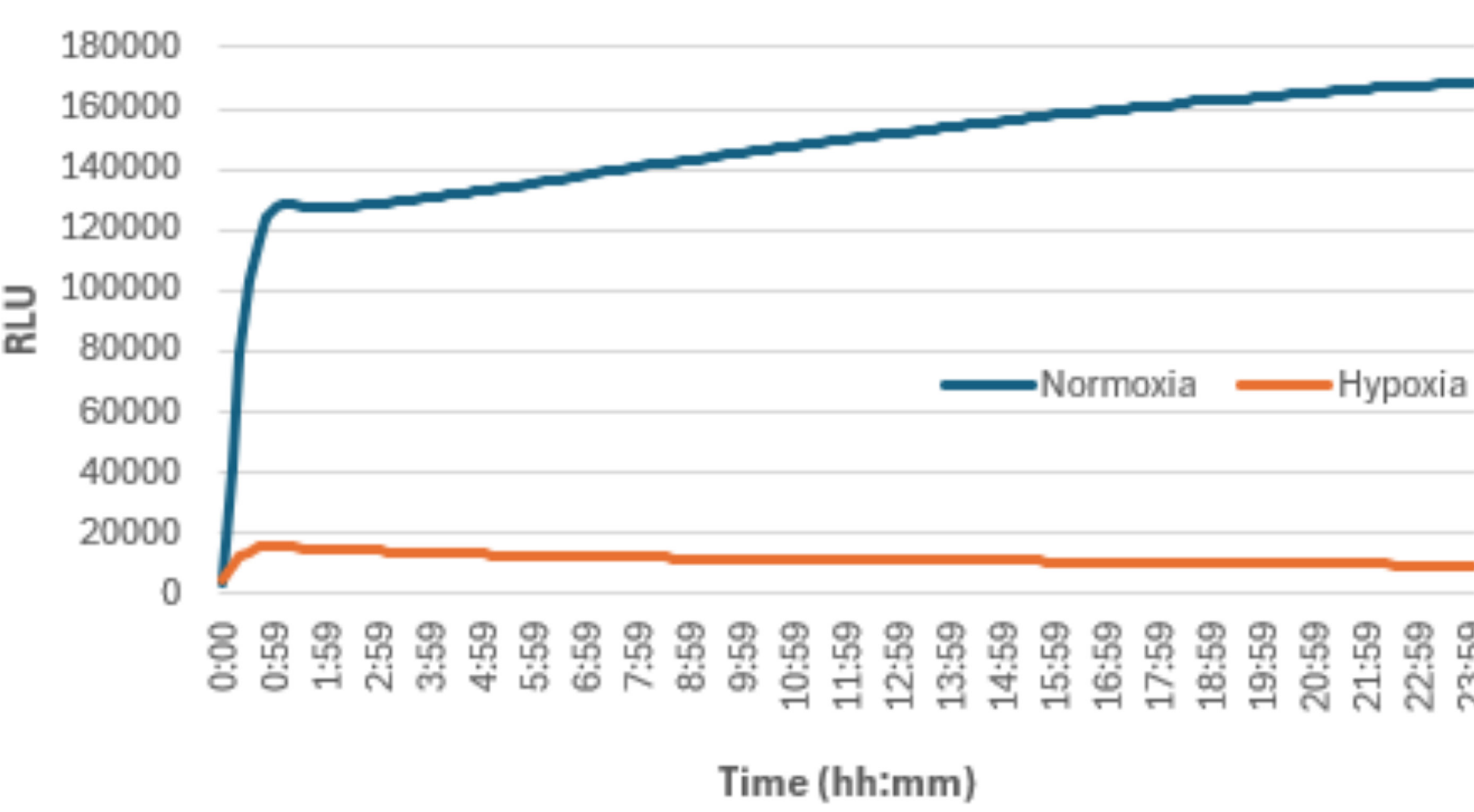


Figure 3. Cell viability monitored over 24 hours using the RealTime-Glo MT assay. Cells were maintained under normoxic or hypoxic conditions, and luminescence was measured every ten minutes, in the SpectraMax iD5e reader.

Effect of GLUT1 inhibitors on uptake measured

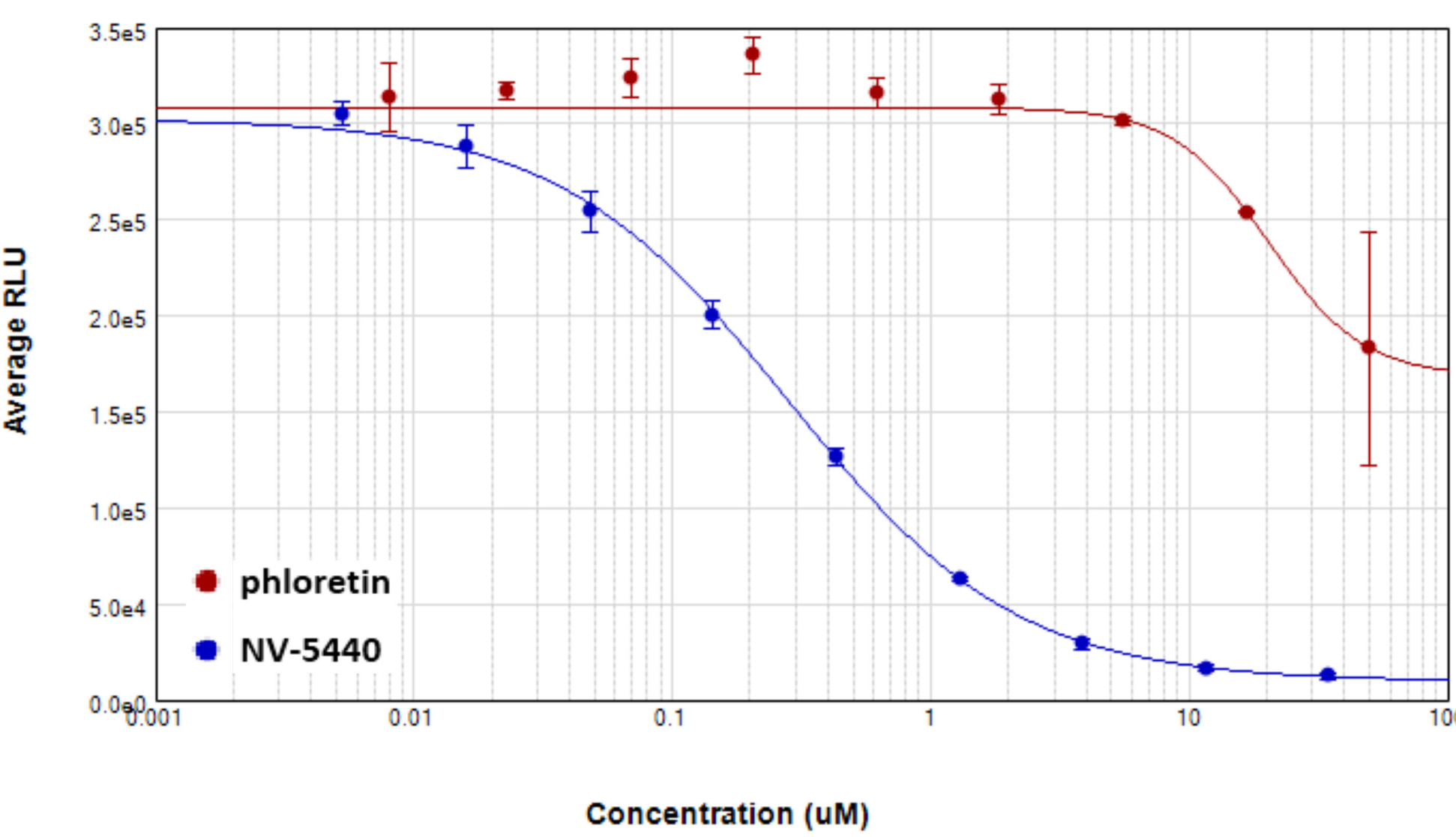


Figure 4. Concentration-dependent inhibition of glucose uptake by the glucose transporter GLUT1 inhibitors phloretin and NV-5440

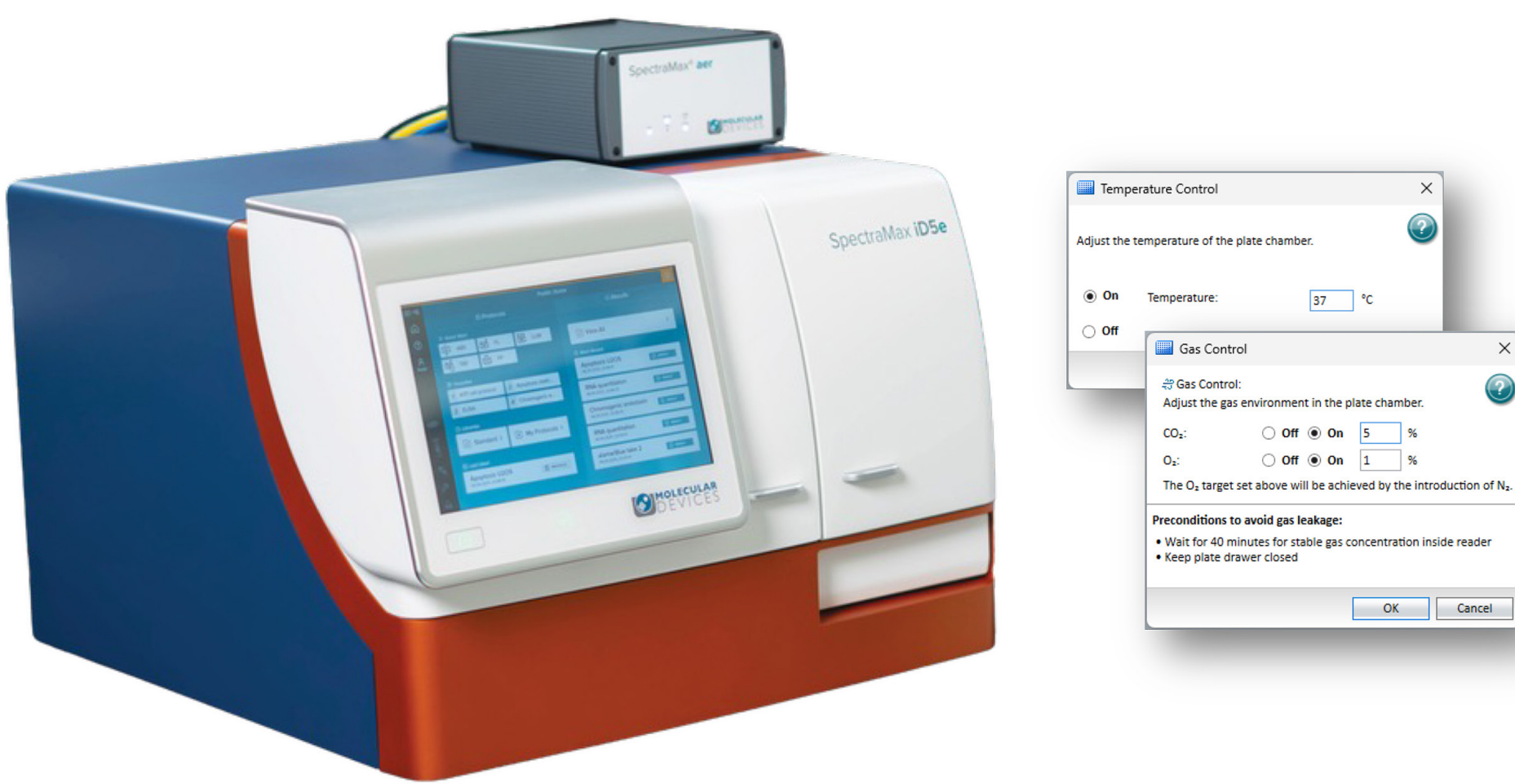


Figure 5. SpectraMax iD5e microplate reader with SpectraMax aer Gas Mixer.

Conclusion

- SpectraMax iD5e reader provides an all-in-one system for maintaining cells under experimental conditions and taking measurements during cell treatment
- SpectraMax aer gas mixer offers environmental control that can mimic conditions, including hypoxia, encountered by cells in different physiological conditions
- Luminescence-based assays give researchers a sensitive and quantifiable way to monitor cell viability and biological responses to metabolic changes