Novel Bioluminescent Cell Metabolism Assays Integration with HP D300 Digital Dispenser and Tecan Gas Controlled Module Equipped Infinite M200 Pro Reader.

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1. Introduction

Robustness of bioluminescence assays makes them attractive and easily adaptable for automation and HTS applications. We describe the integration of Tecan, Hewlett Packard instrumentation capabilities with two of Promega’s live cell metabolism assay formats. The integration is made possible by the use of a novel proluciferin substrate for diaphorase enzyme that is combined with NAD or NADP specific cycling enzymes to develop three assay formulations: 1) Bioluminescence Live Cell Viability Assay, and 2) NAD(P)H/NAD(P) Detection Assays.

2. Advantages Offered by Live Cell Bioluminescence Viability Assay

- Sensitivity and Robustness
  - The most sensitive assay in the market: LOD ~0.5 nM
  - By combining Glowbiomass assay and AlamarBlue for an IC50 determination
  - High robustness of bioluminescence assays makes them attractive and easily adoptable for automation and HTS

3. Continuous monitoring of drug induced changes in cell viability

- Bioluminescence Live Cell Viability Assay allows continuous monitoring of changes in viability in real time
  - Tecan’s Infinite M200 PRO multimode reader with Gas Control Module (GCM) enables long-term kinetic measurements and takes full advantage of the capabilities offered by the live cell viability assay
  - Drug treated cells treated with increasing bortezomib concentrations
  - Time dependent changes in cell viability and IC50 values are determined from the same well

4. Bioluminescent NAD, NADH, NADP and NADPH Detection

A novel proluciferin substrate for diaphorase enzyme was combined with NAD or NADP specific cycling enzymes to develop three assay formulations:

- Measures phosphorylated
  - The luminescence output generated by live cells is correlated with the amount of viable cells
- Measures non phosphorylated

5. Advantages: Ease of Use with Improved Sensitive and Robustness

- AlamarBlue and Tecan Gas Controlled Module Equipped Infinite M200 Pro Reader.
  - The sensitivity (LOD~1nM) and large signal window (S/B>300) of the assays enable measurement of the
- Continuous monitoring of drug induced changes in cell viability
  - Targeting and spaced doses results in more precise EC50 determination
  - Titrations normalized to DMSO.
  - Fast and intuitive processing program.
  - Production of compounds - dead time ~24h

6. Automation And Multiplexing: Experimental Outline

- ADM: Evaluate effect of Iising: an electron transport chain inhibitor and PK866: a known inhibitor of NAD Bioenergetic pathway, on cell viability and NAD/NADH levels
  - Place K562 cells (2k cells/well) containing Cell Viability Reagents added to the media (by Thermocool, J.L.)
  - Read luminescence to determine the amount of viable cells/well to control for plating reproducibility
  - Add inhibitors using HP D300 Dispenser
  - Plate in M200 multimode reader
  - Read luminescence every hour
  - Include 24h
  - Add 30uL NAD/NADH Detection Reagent
  - Read luminescence

7. Automation And Multiplexing: Results

- No decrease in cell viability
  - Rapid decrease in NAD/NADH levels
  - S/B >120
  - % Control
  - Luciferase
  - AlamarBlue
  - AlamarBlue-bc

8. Advantages Offered by Digital Dispenser

- Better results reproducibility - %CV:
  - Significant time savings preparing one 384 plate by direct titration ~2.5min. vs. ~10 min. by Serial Dilutions.
  - Dispense any dose to any well. Fast, targeted and spaced doses results in more precise EC50 determination.
  - Titrations normalized to DMSO.
  - Fast and intuitive processing program.
  - Production of compounds - dead time ~24h

9. Conclusions

- Two novel bioluminescence approaches were developed to monitor cellular metabolism pathways
  - Live cell viability assay is based on measuring the metabolic activity of live cells
  - A novel bioluminescent sensing system is added directly into cell culture medium
  - The luminescence output generated by live cells is correlated with the amount of viable cells
  - The assay is sensitive (detects less than 10 cells/well) and has a wide dynamic range (S/B>100)
  - The unique feature of the assay is the ability to continuously monitor changes in cell viability in real time with multiple measurements performed from the same well
  - Tecan’s Infinite M200 PRO multimode reader with Gas Control Module (GCM) enables long-term kinetic measurements and takes full advantage of the capabilities offered by the live cell viability assay
  - To address the need for rapid and robust measurement of adenine dinucleotides, three assay formats were developed based on the use of a novel proluciferin diaphorase substrate:
  - The assays are easy to use (add and read) and are amenable to HTS screening (Z>0.8)
  - The sensitivity (0.5-1nM) and large signal window (S/B>300) of the assays enable measurement of the
  - The HP D300 Digital Dispenser, available from Tecan, provided rapid and convenient dispensing of compounds and was successfully applied for assay validations

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