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Prep Sheet

Measuring Cell Viability with Promega® CellTiter-Glo

Authored by Kennedy Bae, Ph.D.

Getting Started

Before teaching the lesson plan, complete the following steps prior to class.

- ☐ Setup an OpentronsAI account at opentrons.ai
  - ☐ Note: OpentronsAI must be accessed through Chrome browser
- ☐ Run through the process of prompting OpentronsAI to write a protocol and ensure you are comfortable with the process

Need Additional Support?

For technical support, please check our Opentrons Help Center for relevant articles. If you need further support, please contact support@opentrons.com.

If you have questions related to the lesson plan, please reach out to the author, Kennedy Bae, at kennedy@opentrons.com.

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Educator Guide

Measuring Cell Viability with Promega® CellTiter-Glo

Authored by Kennedy Bae, Ph.D.

Purpose

This lab aims to develop student understanding of using the Promega CellTiter-Glo® kit to measure cell viability in mammalian cells as a method for screening compound toxicity. This includes:

- Cell culture
- Measuring cell viability
- Data analysis and interpretation
- Understanding the relationship between compound concentration and cell viability

Student Audience

This lab is designed for use in mid to upper-level undergraduate cell biology or toxicology courses. It is flexible to accommodate any number of students enrolled in the class.

Background Knowledge

Students should have functional mastery of basic cell culture techniques; understand the principles of luminescence and how the CellTiter-Glo® assay works; proficiency at data analysis using standard curves and statistical analysis.

For understanding the principles of bioluminescence and how the CellTiter-Glo® assay works, see the appended document “Introduction to Bioluminescence”

Core Competencies

Laboratory Skills

- Pipetting, sample preparation, and handling mammalian cell cultures

Critical Thinking

- Dose-dependent effects of compounds on cells
- Interpretation of experimental data, troubleshooting, and drawing reasonable conclusions

Supplies

Opentrons Supplies

- ☐ Opentrons OT-2
    - Note: The protocol associated with this lesson plan can only be run on app and robot server versions 7.3.0 or higher.

- [ ] P1000 single channel GEN2 Pipette (left mount)
- [ ] Opentrons OT-2 96 Tip Rack 1000 µL
- [ ] Opentrons Heater-Shaker
- [ ] Opentrons 96 Flat Bottom Heater-Shaker Adapter
- [ ] Opentrons 10 Tube Rack (6 x 15 mL, 4 x 50 mL)
- [ ] Opaque wall 96-well plate that is compatible with your luminometer

Reagents

- [ ] CellTiter-Glo® Kit
- [ ] Compound for testing
    - Note: we recommend a compound with a 24 hour treatment time.
- [ ] Solvent for compound
- [ ] Cell line selected for testing

Non-Opentrons Equipment

- [ ] Plate reader capable of measuring luminescence
- [ ] Tissue culture infrastructure (sterile hood, incubator, reagents, etc)

Experimental Duration

Required Class Sessions

1-3 depending on whether students are responsible for plating and treating the cells, in addition to the readout components. The below lab is structured as Day 1 (cell plating), Day 2 (treatment), and Day 3 (readout).

Note: The protocol associated with the lesson plan is for the final addition of CellTiter-Glo to the cells. You can adjust the previous portions of this lab and still use the protocol.

Lab Run Time

Cell plating: 20 minutes

Cell treatment: 1 hour

CellTiter-Glo assay and readout: 2.5 hours

- Introduction and setup: 30 minutes
- Compound treatment and incubation: 1 hour
- Cell viability assay and data collection: 1.5 hours

Basic Troubleshooting

1. Do a trial run before class to identify and resolve any unexpected occurrences before students arrive.
2. Confirm cell health before starting the experiment. Suboptimal cell conditions can affect results.
3. Ensure the plate reader settings are optimized for luminescence detection.

Procedure Guide

Day 1 - Cell Culture Preparation ~ 20 minutes

1. Plate cells in a 96-well plate with opaque walls the day before the lab session to allow cells to adhere and grow to the desired confluency. Ensure the plates are compatible with the luminometer used.

Day 2 - Compound Treatment ~ 1 hour

1. Prepare serial dilutions of the compound to be tested in culture media or DMSO.
2. Treat cells with different concentrations of compounds (e.g., 0, 10, 50, 100, 500 µM). The total volume of liquid in each well, post-treatment, should be 100 µL.
    a. Note: We recommend ordering the treatments by column.

3. Incubate cells for the required time (we recommend a compound with 24 hour treatment).

Day 3 - Readout and Analysis ~ (2 hours, 15 minutes)

Pre-lab: CellTiter-Glo Substrate and CellTiter-Glo Buffer Preparation ~ 15 minutes

- Thaw the CellTiter-Glo® Buffer and equilibrate to room temperature prior to use. For convenience, the buffer can be stored at room temperature for up to 48 hours prior to use.
- Equilibrate the lyophilized CellTiter-Glo® Substrate to room temperature.
- Transfer the appropriate volume of CellTiter-Glo® Buffer into the amber bottle containing CellTiter-Glo® Substrate to reconstitute the lyophilized enzyme/substrate mixture. This forms the CellTiter-Glo® Reagent.
- Mix by gently vortexing, swirling, or inverting the contents to obtain a homogeneous solution. The CellTiter-Glo® Substrate should dissolve easily in less than 1 minute
- You will use 11 mL of the CellTiter-Glo® Reagent per robot running this protocol.

Lab Introduction ~ 15 minutes

- Discuss the purpose and principle of the CellTiter-Glo® assay.
- Review pipetting techniques and cell culture handling.

- Explain the importance of controls in assessing cell viability.

Cell Viability Assay ~ 1 hour

1. Equilibrate the plate and its contents at room temperature for approximately 30 minutes.
2. While the plate is equilibrating, setup the robot deck for the protocol, using the below image as your guide.
    a. Note: the 96-well plate of treated cells will be on top of the heater-shaker module.
3. Run the protocol titled “Measuring Cell Viability with Promega® CellTiter-Glo”
    a. This protocol can be downloaded from https://library.opentrons.com/p/promega_cell_viability
    b. The protocol will add the CellTiter-Glo® Reagent to each well in the 96-well plate. This protocol includes two minutes of mixing the reagent with the media, followed by an 8-minute pause. At the end of the protocol, the plate is ready for immediate measurement of luminescent signal.

Data Collection and Analysis ~ 30 minutes

1. Record luminescence using a luminometer. An integration time of 0.25–1 second per well is recommended as a guideline, however you should refer to the recommendations from your luminometer manufacturer.
2. Generate a standard curve using ATP standards (optional).
3. Analyze data to determine the viability of cells at each compound concentration.
4. Use statistical analysis to assess the significance of the results.

Clean up ~ 30 minutes

1. Clean workstations and dispose of waste.
2. Briefly introduce the lab report requirements and provide students with guidelines for data presentation and analysis.

Lab Report

Instructions

Assign students to prepare a comprehensive lab report that includes:

- **Introduction**

    - Background on cell viability assays and the importance of screening compound toxicity.
- **Methods**
    - Detailed protocol followed during the experiment.
- **Results**
    - Raw luminescence data.
    - Standard curve (if ATP standards were used).
    - Graphs showing cell viability as a function of compound concentration.
    - Statistical analysis of the data.
- **Discussion**
    - Interpretation of results.
    - Potential sources of error and variability.
    - Comparison with literature data.
    - Conclusions about the effects of the tested compounds.

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Student Guide

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