RASTRUM

Protocol

In Situ CellTiter-Glo® 3D Assay for RASTRUM™ Advanced Cell Models

Document number | WET.BCM.0012.PRO.v1.03
Date of issue | May 2023

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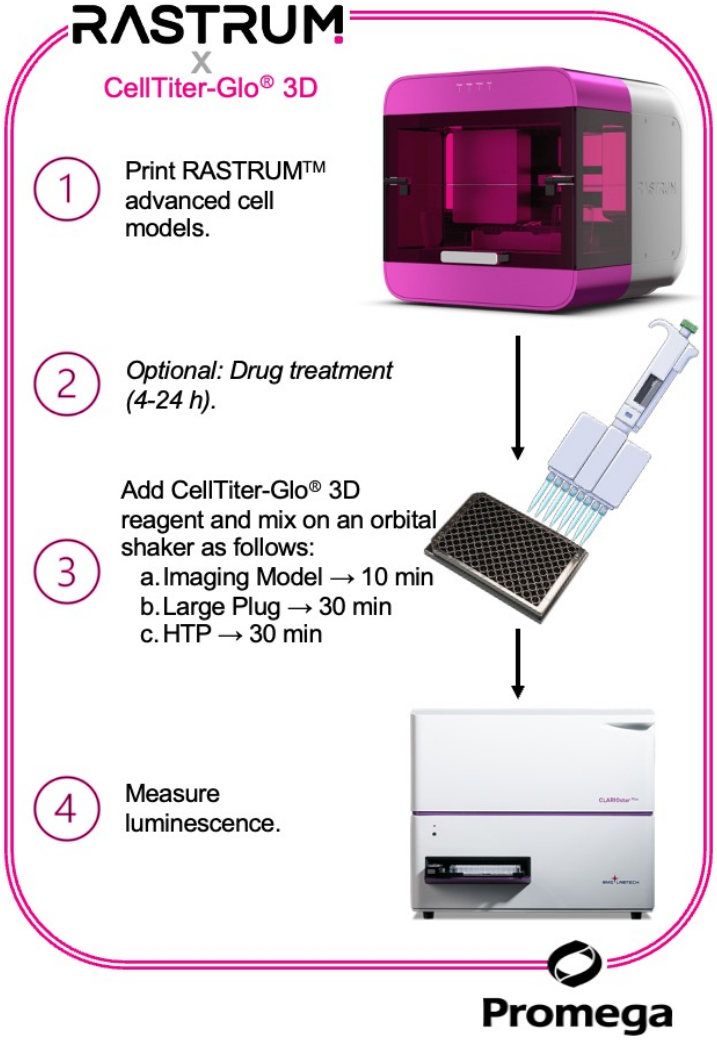
Introduction

Assessing the viability of cells within RASTRUM advanced cell models is a fundamental practice for determining the health of 3D cell cultures. Herein, we have developed a simple luminescent protocol using CellTiter-Glo® 3D (CTG) that permits the assessment of relative cell viability in RASTRUM advanced cell models.

Equipment and reagents required, but not provided

- RASTRUM advanced cell models
- Black-wall, clear-bottom, multiwell plates (PerkinElmer; 6055300 or Greiner; 655087)
- CellTiter-Glo® 3D (Promega; G968, CTG)
- Microplate reader
- Orbital shaker
- Multichannel pipette
- *Optional: Water bath set to 22°C*

Graphical Protocol



Protocol

Note: This protocol can be performed inside or outside a biosafety cabinet.

1. Thaw CTG 3D overnight at 4°C.

   Note: Do not thaw CTG by placing the bottle in the water bath as the bottle may break.

2. Equilibrate the thawed CTG 3D reagent and RASTRUM advanced cell models to room temperature (RT) as follows:

   a. For CTG 3D, equilibrate to RT by warming in a 22°C water bath for ~30 min. Mix gently by inverting the contents to obtain a homogeneous solution.

      Note: CTG 3D can be equilibrated to RT on the benchtop; however, this will require ~45 min–1 h.

   b. For RASTRUM advanced cell models, equilibrate the multiwell plates to RT by incubating them on the benchtop for 30 min.

3. Add an equal volume of CTG 3D to the volume of cell culture medium present in the multiwell plates containing RASTRUM advanced cell models (e.g. for a 96-well plate, add 100 µL of CTG 3D to wells containing 100 µL of cell culture medium).

   Note: We recommend a minimum total liquid volume of 100 µL and 60 µL per well for 96- and 384-well plates, respectively. These volume recommendations are independent of the model printed.

4. Incubate the multiwell plate on an orbital shaker (500 rpm) at room temperature as indicated below to reach luminescent plateau:

   a. Imaging Model: 10 min.

   b. Large Plug: 30 min.

   c. High-Throughput: 30 min.

   Note: Mixing is very important for effective extraction of ATP from 3D microtissues.

5. Measure luminescence using a microplate reader.

   Note: Detection instrument settings vary between devices. Ensure your instrument is enabled for luminescence detection and use an integration time of 0.25–1 second per well as a guideline.

   Note: An uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

Note: Since there are differences in the efficiency of cell lysis between 2D cell cultures and RASTRUM advanced cell models, 2D standard curves are not accurate for quantifying the viability of RASTRUM advanced cell models.

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