

Real-Time Cytotoxicity Analysis Using the CellTox™ Green Assay

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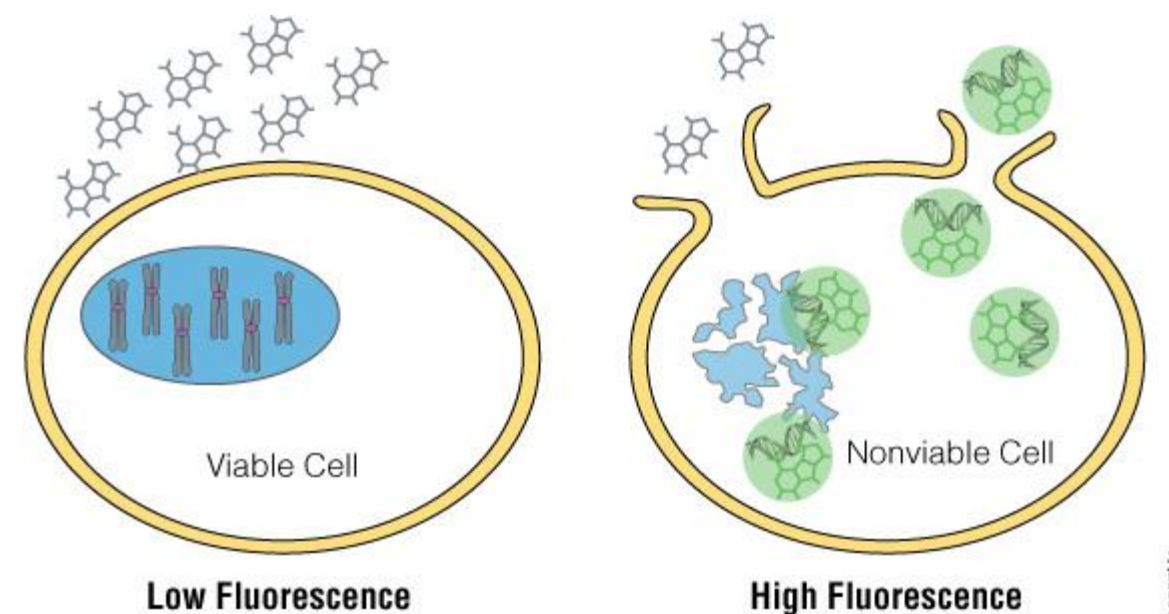


1. Introduction

In vitro cytotoxicity is largely influenced by test article concentration and the exposure period with cells. The diversity of kinetic response can complicate conventional cytotoxicity endpoint assay determinations because most assay reagents are formulated to measure enzymatic biomarkers that are susceptible to time-dependent decay.

Here we demonstrate the use of a non-activity-based cytotoxicity probe, CellTox™ Green Dye, that can be added at the beginning of the experiment and employed in real time for the kinetic determination of cytotoxicity. We used the Tecan HP D300 Digital Dispenser for non-contact dispensing of test compound, and the Tecan Infinite® 200 PRO with GCM™ for kinetic measurement of cytotoxicity over 72 hours.

Figure 1. CellTox™ Green Cytotoxicity Assay. CellTox™ Green Dye is excluded from viable cells, and binds to DNA from cells with compromised membrane integrity.



2. Tecan HP D300 Digital Dispenser

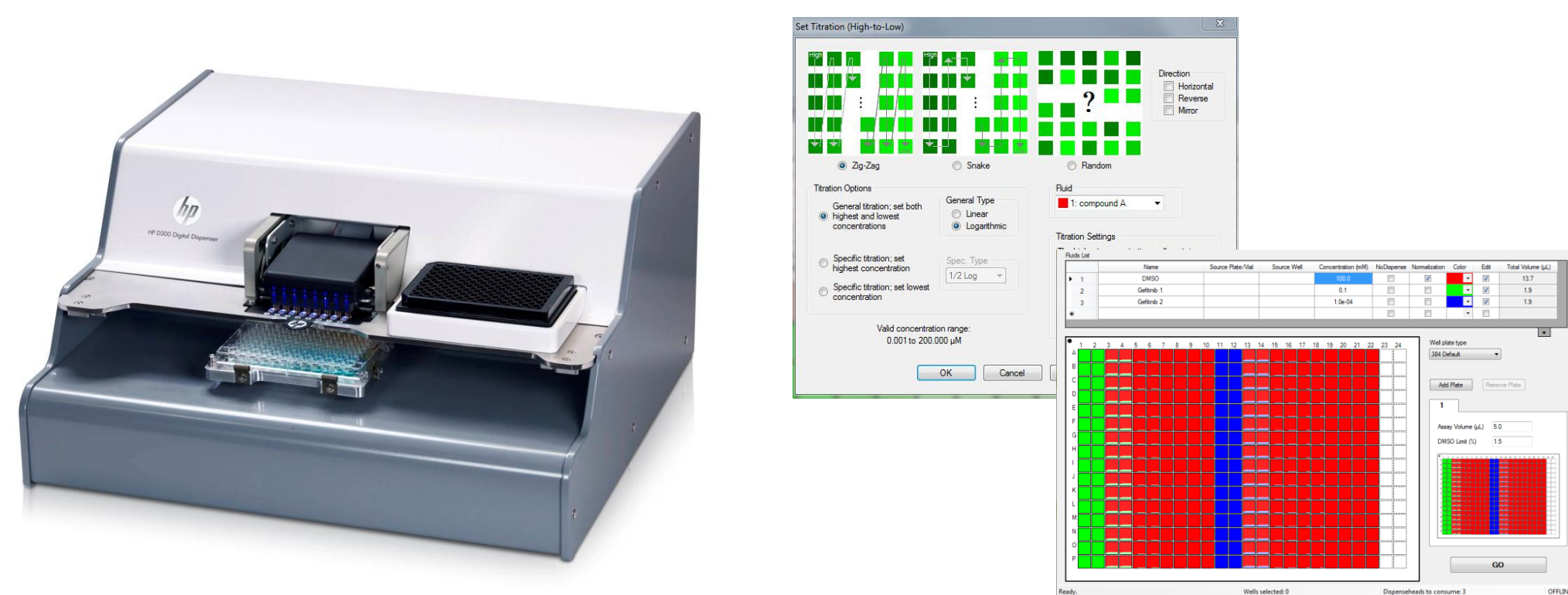


Figure 2. HP D300 Key Benefits.

- Non-contact, direct titration of compound
- Accepts 12- to 384-well plate formats
- Dispense any dose to any well
- Broad volume range: 13µl up to 10µl
- < 2µl dead volume
- Decrease solvent consumption
- Reduce consumable usage

Figure 3. Software Features.

- Fast, easy protocol development
- Flexible dispense patterns
- Randomization to minimize edge effect
- On-the-fly DMSO normalization
- Automatic calculation of dispense head usage

3. Tecan Infinite® 200 PRO with Gas Control Module (GCM™)



Figure 4. Tecan Infinite® 200 PRO with GCM™ Key Benefits.

- Multimode reader with on-board incubator
- Quad4 monochromator for finely tuned wavelength selection
- Simultaneous, independent adjustment of CO₂ and O₂
- Temperature control from ambient +5°C up to 42°C
- Linear and orbital shaking
- Altitude correction for stable regulation of gas concentration inside the reader chamber
- Consistent physiological conditions for predictable cell growth
- Walkaway operation for multi-day time course studies

4. Experimental Setup

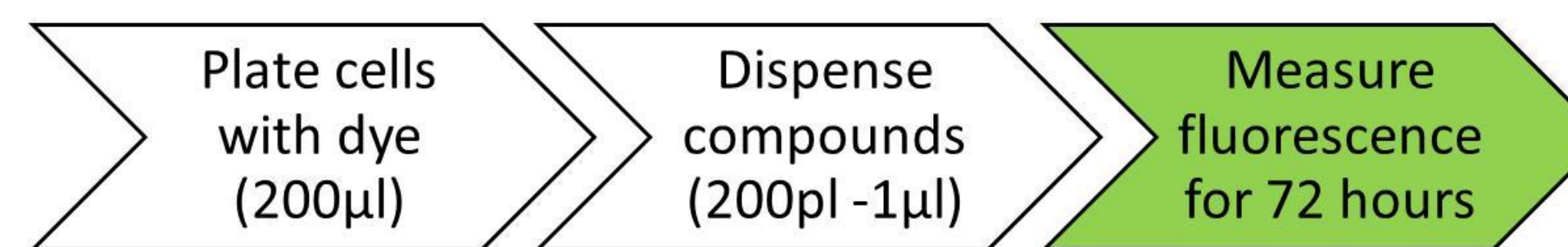


Figure 5. Workflow for performing real-time measurement of cytotoxicity.

Plate setup: Frozen Instant HepG2 cells (Cell Culture Service) were thawed and plated at a density of 10,000 cells/well in 200µl of culture medium containing 1X CellTox™ Green Dye. A black, clear bottom 96-well assay plate (Corning 3603) was used. 150µl of 1X PBS was added to the inter-well spaces. Cells were allowed to adhere for 4 hours prior to compound addition.

Compound dispensing: 200µl - 1µl of test compounds were added with the HP D300. DMSO was backfilled to all wells resulting in final DMSO ranging from 0.1-0.5%.

Plate reader settings: Temperature was set to 37°C with 5% CO₂ and ambient oxygen. Bottom read fluorescence was used; the lid was kept on the plate. All wells were read once every hour for 72 hours using 5 flashes/well with Ex 485(9)nm, Em 520(20)nm and optimal gain regulation. Plate was shaken for 30 seconds (amplitude 2) prior to each read.

5. Real-Time Cytotoxicity Results with HepG2 Cells

Figure 6. Terfenadine exhibits rapid-onset toxicity at high concentrations by a mechanism that is both titratable and time-dependent.

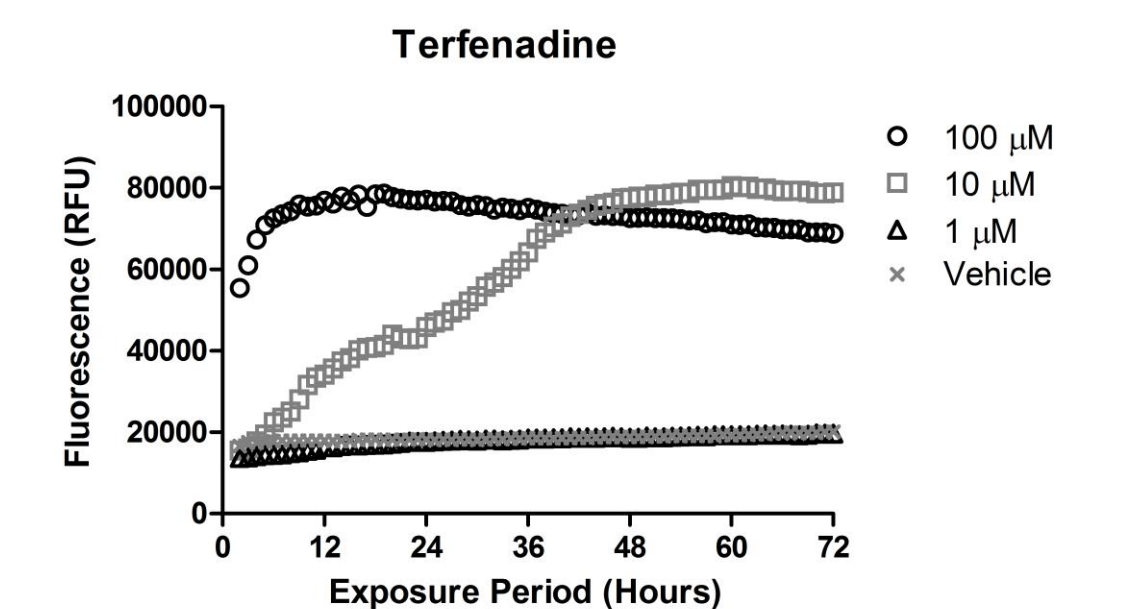
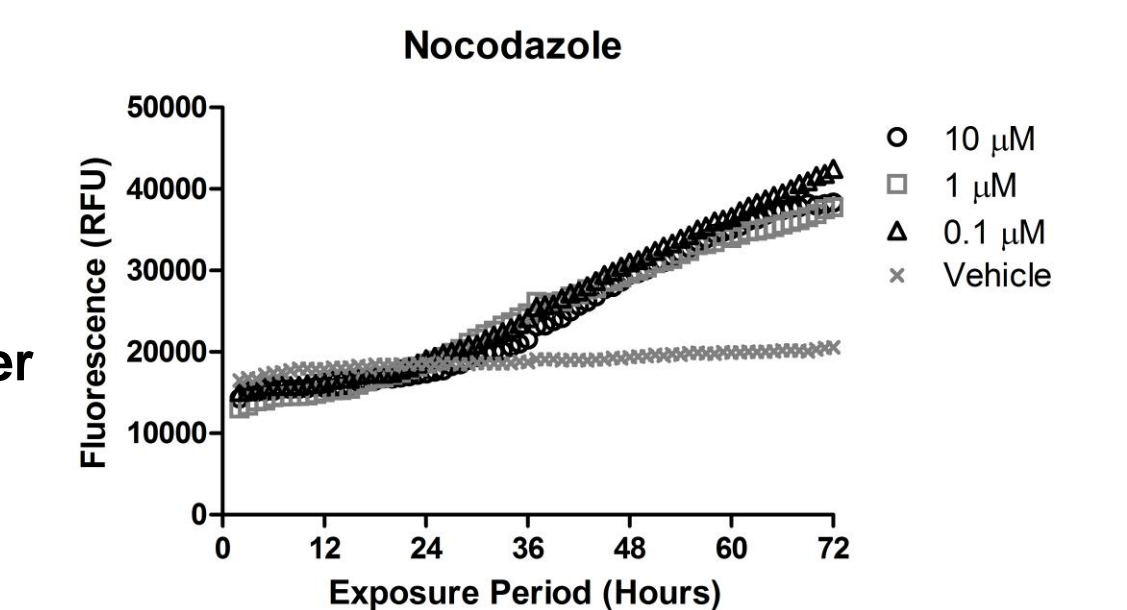


Figure 7. Nocodazole alters microtubule assembly necessary for cell division leading to cytotoxicity beginning at 24 hours with equal potency at dosages over two logs of concentration.



6. Summary

- The CellTox™ Green Assay is comprised of a DNA binding dye that can be used for monitoring membrane integrity changes in real-time.
- Using the HP D300 Digital Dispenser for non-contact, direct titration of test compounds provided a convenient, time efficient means of adding test compounds to the cell assay plate.
- The Tecan Infinite® 200 PRO with GCM™ enabled 72 hour monitoring of cytotoxicity with walkaway operation.
- Performing a 72 hour time course in the microplate reader had no negative impact on cell health as indicated by low fluorescence observed in the vehicle controls.

Acknowledgments

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