Application Note 52633

Use of Custom Methods Capability of NanoDrop Spectrophotometers for Toxicology Studies

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NanoDrop Spectrophotometer, Custom Methods, Cytotoxicity, MTS Assay, Quantification, Toxicology

Introduction

Cell proliferation and cytotoxicity assays are commonly used to quantitatively analyze important cellular processes in order to assess disease states, toxicology, and drug efficacy and safety.[1] Many cell proliferation/cytotoxicity assays rely on a tetrazolium dye which is reduced into a formazan product in the mitochondria of metabolically active cells. Spectrophotometric quantification of the formazan dye can be accomplished by measuring absorbance at 490 nm: decreased absorbance reflects lowered mitochondrial activity, and, by inference, decreased cell health.[2] Many colorimetric cell proliferation/cytotoxicity assays are commonly analyzed using UV-Vis plate readers.

Thermo Scientific™ NanoDrop™ spectrophotometers offer an attractive alternative for performing UV-Vis absorbance measurements. They utilize a proprietary sample retention technology that allows UV-Vis absorbance measurements to be carried out on volumes as small as 0.5 µL without the need for cuvettes or plates. This microvolume capability facilitates re- sampling of wells, multiplexing assays and significantly lowers setup and assay development costs. In addition, these instruments come with flexible, user-friendly software that includes pre-configured programs for common applications, as well as a custom method capability (Method Editor). This capability allows for development and sharing of methods tailored for user-specific needs.

The NanoDrop product line includes the NanoDrop 2000/2000c spectrophotometer which allows for single-sample measurements, and the NanoDrop 8000 spectrophotometer which allows for measurements of up to eight samples at a time.

Custom Methods

The NanoDrop spectrophotometer Method Editor allows the scientist to define various parameters of interest when making UV-Vis measurements. These parameters include extinction coefficient, standard curve options, wavelengths of interest, calculated values using those defined wavelengths, and baseline and other correction values. The custom methods can be readily shared electronically, thereby streamlining collaborative efforts.

MTS Assay Custom Method

This study analyzed the rate at which MTS is reduced to formazan in order to establish an optimal assay time. Additionally, the dose-response relationship of amiodarone in HepG2 cells was examined. Amiodarone is a common positive control for hepatotoxicity during safety assessment of drugs in pre-clinical stages.[3,4,5] HepG2 is a human derived liver cell line.

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For this study, the NanoDrop 2000/2000c Method Editor was first used to create a custom method. Based on the absorbance spectrum of the formazan product (Figure 1), an analysis wavelength of 490 nm and a baseline correction of 750 nm were selected as the custom method parameters. A special auto pathlength feature which permits samples over a wide concentration range to be measured was enabled. The measurement range was set to the visible range (350–840 nm) in order to optimize the signal.

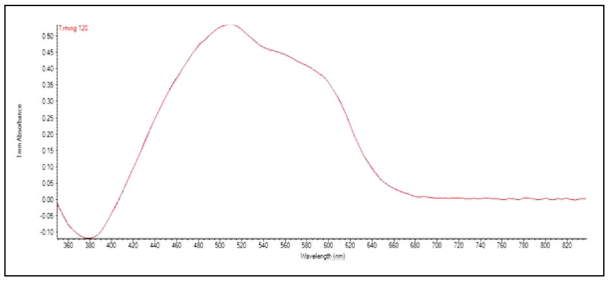

Figure 1. Absorbance spectrum of MTS/formazan. Typical absorbance spectrum of the formazan product when MTS is reduced by metabolically active cells. HepG2 cells were plated at a density of 1.5×10^4 cells/well. After incubation for 48 hours, CellTiter 96 AQueous One Solution reagent (20 µL) was added to cells in 100 µL culture medium. The 96-well plate was incubated at 37 ºC for 105 minutes before absorbance at 490 nm was measured using the NanoDrop 2000/2000c spectrophotometer.

Before commencing the study, a "no cell" control that consisted of culture medium plus the Cell Titer 96 AQueous One Solution Reagent was used to blank the instrument, using the above defined custom method.

The CellTiter 96 AQueous One Solution Cell Proliferation Assay® from Promega® Corp. was selected because it comes in an easy to use format, reducing the number of steps in the assay. This assay kit provides an advantage over the MTT assay because it does not require a solubilization step before measuring absorbance.[6,7]

Rate of MTS Reduction to Formazan

In order to determine optimum assay time, the rate of MTS reduction to formazan in HepG2 cells was monitored by tracking the increase in absorbance at 490 nm over time. HepG2 cells were seeded into 96-well plates and after 48 hours of incubation the MTS assay was performed as described in Figure 2. The absorbance at 490 nm was read on 2 µL aliquots using the NanoDrop 2000/2000c spectrophotometer with the custom method previously described.

Results (Figure 2) show that the HepG2 cells are actively reducing MTS to formazan and that there is a direct correlation between the incubation time and the formazan produced in situ as shown by the steady increase in absorbance over time.

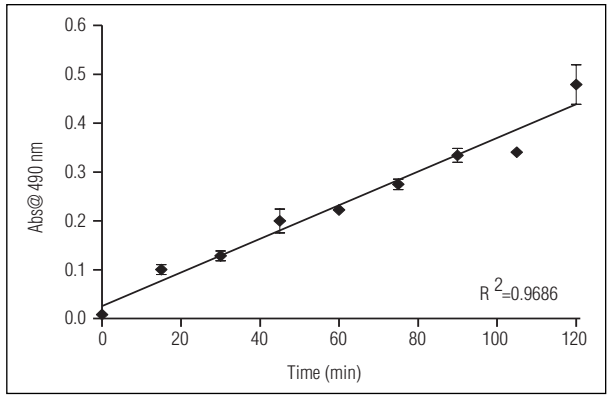

Figure 2: Rate of MTS reduction to formazan. HepG2 cells were seeded in 96-well plates at a density of 1.5×10^4 cells/well and incubated at 37 ºC and 5% CO_2 for 48 hours. The MTS assay was initiated by adding 20 µL of CellTiter 96 AQueous One Solution reagent to each well already containing 100 µL cell culture medium. The rate of MTS reduction to formazan was monitored by removing 10 µL aliquots from each well every 15 minutes for a total of 120 min. For each time point n=4; error bars represent standard deviations.

Amiodarone Effects on HepG2 Cell Viability

HepG2 cell were seeded in 96-well plates and incubated for 24 hours. Cells were then treated with various concentrations of amiodarone and incubated for 24 hours. The MTS assay was performed as described in the Figure 3 legend. As with the MTS reduction study, the absorbance at 490 nm was read using 2 µL aliquots using the NanoDrop 2000/2000c spectrophotometer with the custom method previously described.

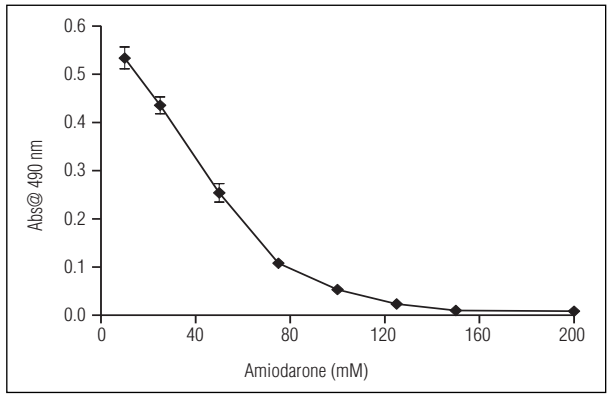

Figure 3. Amiodarone Treatments in HepG2 cells. HepG2 cells were seeded in 96-well plates at a density of 2×10^4 cells/well and incubated at 37 ºC in 5% CO_2 for 24 hours. Cells were treated with various concentrations of amiodarone and further incubated for 24 hours. The MTS assay was initiated as previously described and the plate was incubated at 37 ºC for 90 min. Aliquots (20 µL) were removed from each well after gentle mixing of the reaction before the reaction was stopped by adding 5 µL of 10% SDS. Absorbance at 490 nm was read using 2 µL aliquots on the NanoDrop 2000/2000c spectrophotometer with the custom method described above.

When HepG2 cells were treated with amiodarone, a dose-dependent decrease in absorbance was detected (Figure 3), similar to that observed in previously published reports.[8] Our data verifies that amiodarone decreases HepG2 cell viability and thus, it is cytotoxic to these cells. The data presented here show that the NanoDrop 2000c spectrophotometer and accompanying Method Editor presents a versatility that is highly valuable in cytotoxicity experiments, especially during method development.

Conclusions

This study demonstrates the use of the Thermo Scientific NanoDrop 2000/2000c spectrophotometers' custom methods capability to perform colorimetric cell proliferation/cytotoxicity assays. The instrument provides for a user-friendly way to tailor multi-parameter assays, even when optimum absorbance wavelengths are unknown, and simple data collection and analysis. The NanoDrop 2000/2000c spectrophotometer may be especially useful to researchers that perform small scale experiments such as optimization of assay conditions because the small sample volumes needed for readings allow for repeated sampling from the same well. As researchers gather more information on mechanisms of cytotoxicity by performing multiple assays on the same population of cells, the ability to carry out readings with reduced sample volumes can be highly valuable.

References

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