MOLECULAR DEVICES

APPLICATION NOTE

Delfia cell proliferation assay on the SpectraMax i3 Multi-Mode Microplate Reader

Benefits

- **Sensitive detection of cells down to ~34 cells per well**
- **Wide dynamic range of cell densities over 3 decades**
- **Preconfigured protocol in SoftMax Pro Software**

Introduction

Cell proliferation is an important parameter to measure when testing compound toxicity or the effect of cytokines on a population of cells. Since cell proliferation requires the synthesis of DNA, one accurate way to measure cell proliferation is to monitor the uptake of the modified thymidine nucleotide BrdU[1]. The DELFIA® cell proliferation assay is a time-resolved immunoassay based on the incorporation of BrdU into newly synthesized DNA strands of proliferating cells.

BrdU is added to cells cultured in a microplate, where it is then incorporated into the DNA of proliferating cells. Incorporated BrdU is detected using a europium-labeled monoclonal antibody. Cells are fixed and DNA is denatured. Then, unbound antibody is washed away, DELFIA inducer reagent is added, and europium is dissociated from the labeled antibody into solution, forming highly fluorescent chelates. Fluorescence is proportional to the amount of DNA synthesis in each well's cell population. Time-resolved detection minimizes background fluorescence and compound interference, enabling greater sensitivity than other fluorescence-based cell proliferation methods.

In this application note, we used the DELFIA cell proliferation assay to assess cytotoxicity of the chemotherapy drugs paclitaxel and etoposide in HeLa cells using the SpectraMax® i3 Multi-Mode Microplate Reader.

Materials

- DELFIA Cell Proliferation Assay Kit (Perkin-Elmer cat. #AD0200)
- HeLa cell line (ATCC cat. #CCL-2)
- Complete culture medium for HeLa
  - FBS (Gemini Bio-Products cat. #100-106)
  - Penicillin-streptomycin (Thermo Fisher Scientific cat. #15070-063)
  - 1x MEM (Corning cat. #10-009-CV)
- 96-well, clear-bottom, tissue culture-treated microplates (Corning cat. #3904)
- SpectraMax i3 Multi-Mode Microplate Reader
  - SpectraMax Time-Resolved Fluorescence (TRF) Detection Cartridge (Molecular Devices cat. #0200-7008)

Methods

HeLa cells grown to 90% confluence were trypsinized, counted, and seeded into a black 96-well clear-bottom tissue-culture microplate at 10,000 cells per well. Cells were allowed to attach and grow overnight and then treated with serial dilutions of paclitaxel or etoposide for 48 hours. The DELFIA cell proliferation assay was performed as described below. For more details, please refer to the technical data sheet for the kit[2].

To determine the dynamic range of the DELFIA cell proliferation assay, HeLa cells were seeded at densities ranging from 11 to 75,000 cells per well. Labeling solution was added immediately upon cell seeding, and the assay was performed as described below.

Reagent preparation

- BrdU Labeling Solution at 100 µM was prepared by diluting BrdU Labeling Reagent 1: 100 with sterile culture medium. The resulting solution was protected from light.
- Anti-BrdU-Eu working solution at 0.5 µg/mL was prepared by diluting the 100 µg/mL stock solution 1:200 with Assay Buffer.
- Wash Concentrate was diluted 25-fold with distilled water to make Wash Solution.

Assay procedure

- Cells were labeled with 1/10 volume of the 100 µM Labeling Reagent, e.g. 20 µL if cells were cultured in 200 µL of medium, and incubated for 24 hours in a 37°C incubator.
- Labeling medium was removed from the wells, and 100 µL of Fix Solution was added to each well. The plate was incubated for 30 minutes at room temperature on an orbital shaker (~100-120 rpm).
- Fix Solution was removed thoroughly from the wells. 100 µL of 0.5 µg/mL Anti-BrdU-Eu working solution was added to each well, and the plate was incubated for 60 minutes at room temperature on an orbital shaker.
- Anti-BrdU working solution was removed, and the wells were manually washed with 300 µL Wash Solution per well. 200 µL of DELFIA Inducer was added to each well.
- The plate was incubated at room temperature for 15 minutes on an orbital shaker, and then europium fluorescence emission was measured.

Optimized settings for the SpectraMax i3 reader (Table 1) were used to detect the assay. Results were analyzed and graphed using a preconfigured protocol in SoftMax® Pro Software.

Results

HeLa cells treated with the chemotherapy drugs paclitaxel and etoposide exhibited IC_{50} values comparable to those reported in the literature (Figure 1)[3,4]. The IC_{50} value for paclitaxel (Taxol) was 1.4 nM, while the topoisomerase II inhibitor etoposide was much less potent, with an IC_{50} value of 0.81 µM.

Parameter	Setting
Optical configuration	TRF Detection Cartridge
Read mode	TRF
Read type	Endpoint
Wavelengths	Ex 370 nm Em 616 nm
PMT and Optics	Number of pulses: 100 Excitation Time: 0.05 ms Measurement Delay: 0.25 ms Integration Time: 0.4 ms Read Height: 2.36 mm

Table 1. Instrument settings for DELFIA cell proliferation assay. The detection cartridge includes excitation light source and optics as indicated. Optimized PMT and Optics settings vary slightly from values listed in the technical data sheet for the kit and were optimized for the SpectraMax i3 reader.

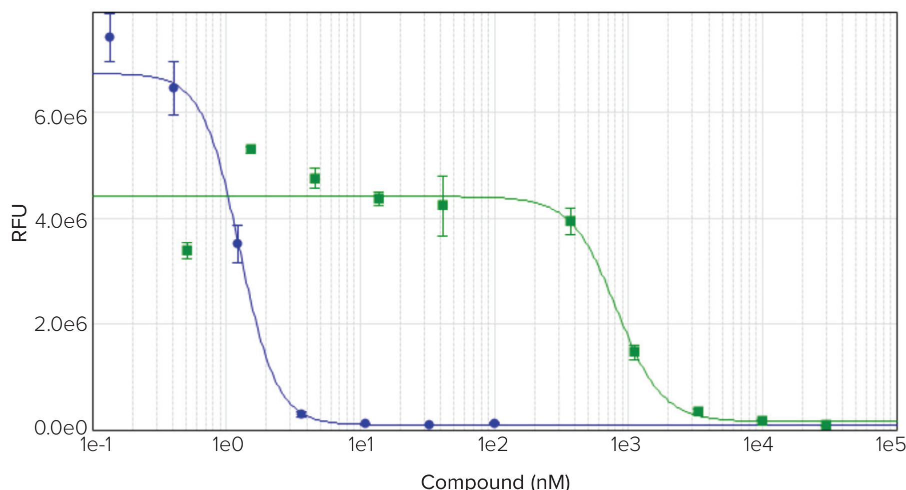

Figure 1. HeLa cells were treated with paclitaxel (blue circles) or etoposide (green squares) for 48 hours. Paclitaxel, IC_{50} = 1.4 nM; etoposide, IC_{50} = 0.81 µM. IC_{50} values were calculated in SoftMax Pro Software.

The dynamic range for the DELFIA cell proliferation assay covered about 3.5 decades. Inclusion of a cell-free assay blank allowed for calculation of a lower limit of detection at about 34 cells per well (Figure 2).

Conclusion

As shown in the results above, the DELFIA cell proliferation assay combined with the SpectraMax i3 reader gives researchers a very sensitive test for measuring cell proliferation. The assay can accurately test the toxicology of compounds as shown in Figure 1. The dynamic range of the assay is about 3.5 decades, with sensitive detection of cells down to about 34 cells per well, making this a very versatile assay for use under a variety of cell densities and experimental treatment conditions.

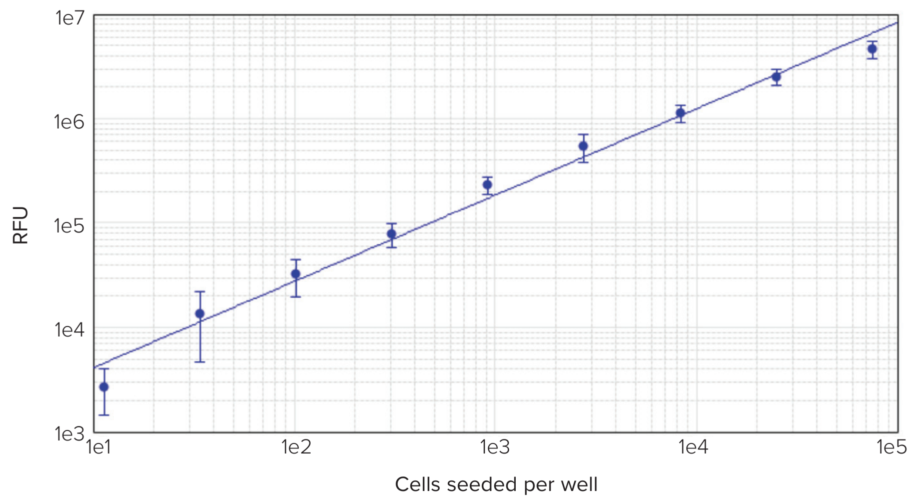

Figure 2. The dynamic range of the assay was assessed by counting a serial dilution of cells. Shown above, the linear range extends from 75,000 cells to about 34 cells per well ($R^2 = 0.99$, n = 8).

References

1. Gratzner HG. 1982. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. *Science* Oct 29; 218(4571): 474-5.
2. Technical Data Sheet: DELFIA Research Reagents, Cell Proliferation Kit.
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4. Lock RB and Stribinskiene L. 1996. Dual Modes of Death Induced by Etoposide in Human Epithelial Tumor Cells Allow Bcl-2 to Inhibit Apoptosis without Affecting Clonogenic Survival. *Cancer Research* 56: 4006-4012.

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