

An Easy Way to Perform Cytotoxicity Assays with Frozen Cells

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Key words

- Cytotoxicity Assays
- Thermo Scientific Multiskan FC
- Microplate Photometer
- CellTiter96 AQ Proliferation Assays
- PRINCESS PIA Assays
- Thermo Scientific SkanIt Software

Abstract

This application note explains how to easily perform a cytotoxicity assay using frozen cells and a Thermo Scientific Multiskan FC microplate photometer.

The assay described in the paper is a cell viability assay from Promega Corporation. The cells used in the assays were deep-frozen, ready-to-use human hepatoma cells.

Introduction

In addition to sensitivity and reproducibility, ease of use is an important assay selection criteria. Choosing the best possible screening platform may be further complicated by additional factors, including cell line, plate setup and the equilibration period.

CellTiter 96[®] AQueous One Solution Cell Proliferation Assay is a homogeneous colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The CellTiter 96 AQueous One Solution Reagent contains a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium,

inner salt; MTS(a)] and an electron coupling reagent (phenazine ethosulfate; PES). PES has enhanced chemical stability, which allows it to be combined with MTS to form a stable solution. The MTS tetrazolium compound is bio-reduced by cells into a colored formazan product that is soluble in tissue culture medium. This conversion is accomplished by NADPH or NADH produced by dehydrogenase enzymes in metabolically active cells. The quantity of formazan product as measured by the absorbance at 490 nm is directly proportional to the number of living cells in the culture.

PRINCESS PIAcells

The PRINCESS[®] PIAcells are ready-to-use cells frozen on a 96-well plate. The instant-proliferation assay has been developed for fast and reliable screening of new drugs with regard to their cytostatic, anti-cancer effect. Human tumor cell lines are frozen on microwell plates without toxic cryoprotectants. The cells can be used immediately after thawing. For routine proliferation assays, different tumor cell lines are prepared in PRINCESS assay plates that can be used with all common viability protocols. The cells used in this study were HepG2 human hepatoma cells.

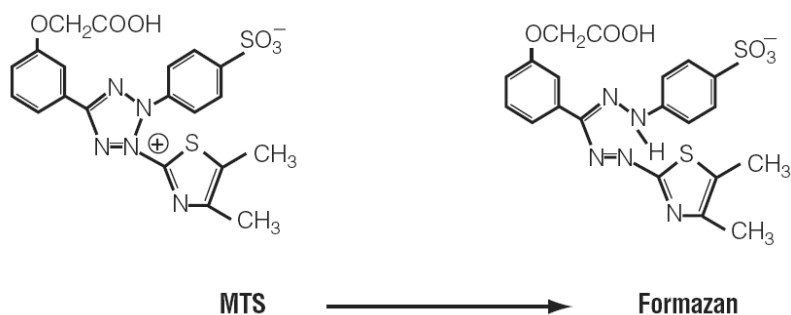


Figure 1. The colorless MTS tetrazolium compound is converted into a colored formazan product.

Materials and methods

- HepG2 cells: PRINCESS PIA instant proliferation assay (Cell Culture Service, Product number PA261-00)
- CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Product number G3582)
- Etoposide (Sigma Aldrich, Product number E1383)
- Polymyxin B Sulfate salt (Sigma Aldrich, Product number P4119)
- 5-fluorouracil, control from the PRINCESS PIA assay (Cell Culture Service, Product number PA261-00)
- Multiskan® FC photometer

The test procedure consisted of three phases:

1. Equilibration: After thawing, the cells were reactivated by adding the culture medium. The plates were then incubated for 48 hours.
2. Exposure: Once the cells were attached, three test compounds were added. An eight fold dilution series of test compounds was added to the plate in duplicates. The final concentrations per well are described in Table I. The negative control (no test compound) was assayed in eight replicates.
3. The plates were then incubated in a CO₂ incubator (37 °C, 5% CO₂) with a humidified atmosphere to induce cell death. The cells were incubated with test compounds for 24 hours in all of the assays.
4. Assay: Celltiter AQ: 20 µl of CellTiter 96 AQueous One Solution Reagent was pipetted into each well. Each plate was incubated for
5. 1.5 hours at 37 °C in a humidified, 5% CO₂ atmosphere. The absorbance

was recorded at 490 nm with the Multiskan FC Photometer.

Compound	Concentration range
5-fluorouracil	0.0045 – 10 mM
Polymyxin	230 – 500000 U/ml
Etoposide	0.005 – 4 mg/ml

Table I. Test compounds

All results were calculated using Thermo Scientific SkanIt Software for the Multiskan FC photometer.

Results

SkanIt® Software has an automatic calculation step for effective dose values. The step creates a graph and calculates the user-defined ED values, for example, ED₅₀. In this assay the ED₅₀ values were calculated for all three test compounds. The values are reported in Table II. An example of a SkanIt Software ED calculation curve is charted in Figure 2.

	Multiskan FC
Etoposide (mg/ml)	0.08
Polymyxin (kU/ml)	3818
5-fluorouracil (mM)	0.18

Table II. values of test compounds with the Celltiter AQ assay

As in most cell assays, the variation between sample replicates was quite high in

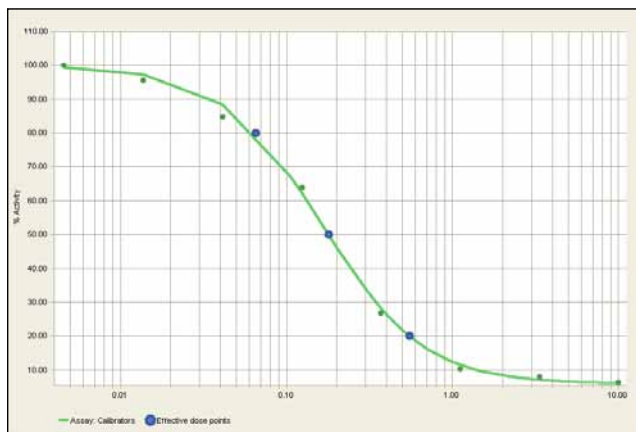


Figure 2. IC curve for 5-fluorouracil. ED-80, ED-50 and ED-20 are marked with blue dots on the curve.

the assay, typically CV > 10%. However, the variation among replicate samples in this case is mainly caused by the cells rather than by the assay chemistry or detection.

Conclusion

This study demonstrates that it is possible to perform the assay with an “instant” cell kit, and is an easy way to start the assay development or optimization process.

The Multiskan FC instrument is shown to be an excellent tool for photometric cytotoxicity studies. The SkanIt Software’s automatic ED (IC) calculation function was shown to be very valuable. Furthermore, neither the assay incubation times, nor plates nor instrument settings were optimized, which likely would have improved the assay performance and precision.

References

CellTiter 96 AQueous One Solution Cell Proliferation Assay, technical bulletin, Promega Corp.

Further Information

For further information about Multiskan FC, please refer to the following Web pages:

- www.thermo.com/readingroom
- www.thermo.com/mpi

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