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Application Note

Quantifying Cytotoxicity of Thiostrepton on Mesothelioma Cells using MTT Assay and the Epoch™ Microplate Spectrophotometer

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The Epoch Microplate Spectrophotometer is the heart of the Epoch Multi-Volume Spectrophotometer System and allows for the practice of a wide variety of colorimetric microplate-based assays. Here we demonstrate the ability to quantify the toxic effect thiostrepton has on mesothelioma cells using Invitrogen's Vybrant® MTT Cell Proliferation Assay Kit.

Introduction

The ability to quantify the response of a cell population to external factors is central to a broad spectrum of research applications such as:

- the toxic effects of drug compounds on primary cell cultures
- analysis of growth factor activity
- screening for environmental toxins
- evaluation of the cytostatic potential of anti-tumor compounds

While several methods exist to determine the viability and proliferation of cells in response to stimuli including microscopic analysis and automated cell counting, indirect methods using fluorometric and colorimetric reagents are currently the most attractive alternatives as assays are rapid and easy to perform. The MTT assay developed by Mossman is a convenient and safe method for determination of live cell number[1]. The MTT assay is a colorimetric assay that relies on the enzymatic reduction of a yellow tetrazolium salt, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), which forms a purple formazan crystal in metabolically active cells (Figure 1). The formazan can then be solubilized producing a concentration dependent colorimetric signal at 570 nm proportional to the cell number and activity. This results in a sensitive assay that is readily adaptable to a 96-well microplate format to analyze a large numbers of samples using a microplate absorbance reader.

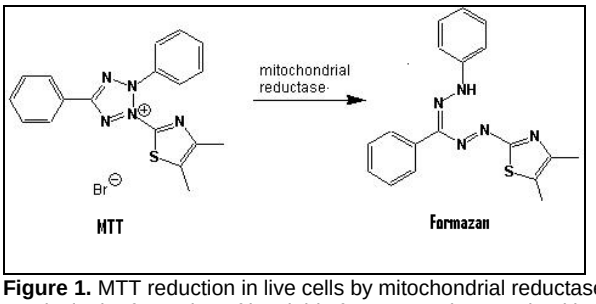

Figure 1. MTT reduction in live cells by mitochondrial reductase results in the formation of insoluble formazan, characterized by high absorptivity at 570 nm.

Invitrogen's Vybrant® MTT Cell Proliferation Assay Kit is a popular choice for accurately performing live cell analysis under a variety of experimental conditions. Here we show that the mesothelioma cellular response to treatment with the cytotoxic agent thiostrepton can be accurately quantified using the Vybrant MTT kit and the Epoch Microplate Spectrophotometer .

Materials and Methods

All measurements were made with the Epoch™ Microplate Spectrophotometer and 96-well microplates. Epoch is also available as a Multi-Volume Multi-Sample Spectrophotometer System (BioTek Instruments, Inc., Winooski, VT) that incorporates the Take3 plate, which allows for micro-volume spectrophotometer quantification of nucleic acids and protein (sample volume: 2 µL), 1 cm path length direct measurements using BioTek's BioCell or any standard spectophotometric cuvette (Figure 2).

Figure 2. The Epoch™ Multi-Volume Spectrophotometer System incorporates the Epoch Spectrophotometer, Take3™ Multi-Volume, Multi-Sample plate and the ability to use standard microplates allowing for a diverse range of research and analytical applications.

To determine the effect of the cytotoxic agent thiostrepton on cell viability, a Vybrant MTT Cell Proliferation Assay Kit (V-13154), culture media and fetal bovine serum was obtained from Invitrogen (Carlsbad, CA). Human primary mesothelioma cells were plated in standard 96-well microplates.

As described previously[2], cells were cultured in DMEM/F12 supplemented with 10% fetal bovine serum (FBS). Cells were trypsinized and re-suspended in fresh media at a concentration of 50,000 cells/mL. Serial dilutions (1:2) were made using DMEM/F12 with 10% FBS as the diluent. Aliquots (200 µL) of each cell dilution were then pipetted into 96-well microplates. Cells were allowed to grow overnight at 37°C in a humidified 5% C_{O2} environment. Cell densities ranged from approximately 50,000 to 100 cells/well with 8 replicates per density prior to treatment with cell counting reagent.

The cells were quantified using the Vybrant® MTT Cell Proliferation Assay per the kit product information sheet. Briefly, prior to addition of the MTT reagent microplates were subject to removal of media by decantation followed by addition of 100 µL of fresh media and 10 µL of freshly prepared MTT reagent. The plates were incubated at 37º C for 4 hours prior to addition of 100 µL freshly prepared SDS-HCl solution followed by mixing with a pipettor. The plates were incubated overnight at 37º C and absorbance read at 570 nm on the Epoch Microplate Spectrophotometer.

The reader was controlled and data analysis performed using the Gen5™ Data Analysis Software package (BioTek Instruments, Inc., Winooski, VT).

For cytotoxic agent studies, primary human mesothelioma cells were seeded at 20,000 cells per well in a 200 µL volume in DMEM/F12 media supplemented with 10% and allowed to grow overnight at 37°C in a humidified 5% CO_2 environment. The cells were then subjected to treatment with thiostrepton, a polypeptide antibiotic, at concentrations ranging from 20 to 0.0195 µM with 8 replicates per concentration and allowed to grow for an additional 24 hours prior to addition of the cell counting reagent. MTT reagent was added, absorbance read and data analyzed as described below.

All absorbance measurements were blank corrected where the blank consisted of media and MTT reagent

Results

A cell titration curve was generated from serial dilution of primary human mesothelioma cells and MTT reagent (figure 3). The raw 570 nm absorbance readings were corrected by subtracting the mean absorbance of the blank wells (media and MTT reagent alone). The standard curve indicates a linear response between cell number and absorption at 570 nm.

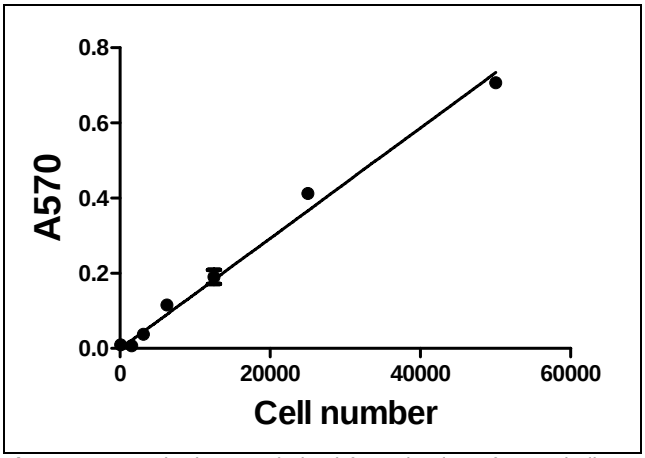

Figure 3. Standard curve derived from titration of mesothelioma cells using Vybrant® MTT Cell Viability Assay.

A dose response of thiostrepton effect on mesothelioma cells is show in figure 4. The cell titration curve was used to generate a thiostrepton dose response curve exhibiting the typical sigmoidal form associated with cell viability assays. The EC50 was calculated to be 3.5 µM under the conditions tested.

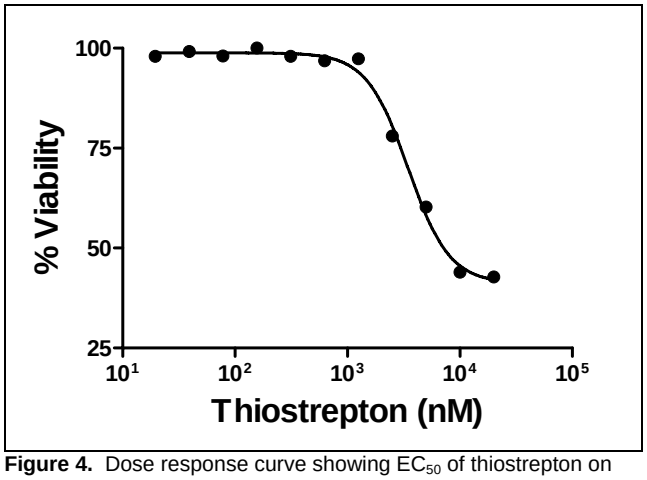

Figure 4. Dose response curve showing EC_{50} of thiostrepton on inhibition of mesothelioma cell viability.

This result compares well with recently published data indicating thiazole antibiotics target FoxM1 inducing apoptosis in various cells lines with concentrations in the low micromolar range (0.7 – 6 µM), as determined by growth inhibitions assays[3].

Conclusion

The MTT assay provides a safe, effective method to quantify cell viability or proliferation in response to a variety of external stimuli. The colorimetric basis of the assay and ease of reagent addition directly to the cell culture makes it easily scalable for use in microplates and absorbance microplate readers. Here we have show that mesothelial cells grown on a microtiter plate and treated with the cytotoxic agent thiostrepton can be quantified using Vybrant MTT and the Epoch Microplate Spectrophotometer.

References

1. Mosmann T (1983) “Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays". Journal of Immunological Methods. **65:** 55–63.
2. Held, P. (2009) “An Absorbance-based Cytotoxicity Assay using High Absorptivity, Water-soluble Tetrazolium Salts”. Application Note. BioTek Instruments, INC., Winooski, Vermont, 05404.
3. Bhat UG, Halasi M, Gartel AL (2009) “Thiazole Antibiotics Target FoxM1 and Induce Apoptosis in Human Cancer Cells”. PLoS ONE 4(5): e5592.