Cell Proliferation Assay Using the Benchmark™ Plus Microplate Spectrophotometer

William C Heiser, PhD and Ma Theresa Redila-Flores, Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

An accurate and rapid assessment of viable cell number and proliferation is often required for in vitro and in vivo experiments. These assessments are necessary and useful for analysis of growth factor activity, drug screening, serum batch testing, and toxicological determination of carcinogenic compounds, to name a few. Cell proliferation kits are used to measure cell viability and division in response to growth factors, cytokines, and nutrients, as well as growth inhibition by cytotoxic agents. The cell proliferation kit described here utilizes a microplate spectrophotometer to quantitate an assay based on XTT, a tetrazolium salt that is cleaved as a result of metabolic activity to generate a colored formazan product.

Method

A cell proliferation kit from Roche was used to measure proliferation of A549 cells grown at different densities overnight. The cells were incubated with XTT before measuring the absorbance. To confirm the optimal wavelength at which to read the samples, a spectral scan was done. The cells were then read after 1, 2, and 3 hr of incubation as suggested in the kit instructions.

Materials

A549 cells	ATCC catalog #CCL-185
DMEM medium	GIBCO BRL catalog #10370-021
Dulbecco's phosphate-buffered saline (PBS)	GIBCO BRL catalog #14287-098
Trypsin-EDTA (0.5%)	GIBCO BRL catalog #15400-096
Penicillin-streptomycin	GIBCO BRL catalog #15070-089
Fetal bovine serum	GIBCO BRL catalog #26140-079
96-well plate, clear polypropylene	Falcon catalog #3072
Cell proliferation kit II (XTT)	Roche catalog #1-465-015
Benchmark Plus microplate spectrophotometer	Bio-Rad catalog #170-6930

Procedure

1. Inoculate A549 cells into a 25 cm^2 flask with DMEM or appropriate medium. Grow cells to 70% confluence.
2. Wash cells with 1x PBS. Trypsinize, then add 2 ml of appropriate medium.
3. Dilute cells 10-fold, to about 2.5 x 10^5 cells/ml. Prepare 2-fold dilutions and plate 100 µl of each dilution in replicates of 5 in a 96-well plate. Prepare a blank by dispensing medium without cells in replicates of 5. Swirl the plate to evenly distribute cells in the wells. Incubate plate overnight at 37°C.
4. Prepare XTT labeling mix according to instructions and add 50 µl to each well. Incubate at 37°C for 1, 2, and 3 hr.
5. After 1 hr of incubation, place the plate into the Benchmark Plus.
6. To determine the optimum wavelength for the assay, run a spectral scan. In Microplate Manager PC software, select File>New Endpoint Protocol>Advanced Options> Scan Well. Scan three wells with different cell densities across the wavelength range 340–800 nm with a step size of 5 nm. Next, construct a graph to show the peak wavelength around 475 nm (the recommended wavelength for reading the assay) for all samples scanned.
7. After the spectral scan, open the Microplate Manager™ software application. Under the File menu, select New Endpoint Protocol. Next, select the 96-well format and edit the template, selecting the wells used. Specify a single-wavelength reading at the peak wavelength determined in step 6.
8. Assay the plate using the Benchmark Plus by clicking the Run button. Repeat for the 2 and 3 hr incubation times.
9. After reading the samples, export or copy and paste the data points into Microsoft Excel software and plot them.

Results

Figure 1 shows the results obtained by scanning sample wells with different cell densities (step 6 of Procedure). The scan resulted in an absorbance curve that peaked at about 475 nm, the wavelength specified for sample reading in the XTT kit instructions.

The assay was performed at 475 nm. Figure 2 shows a proportional increase in the OD value at 475 nm with increasing cell number.

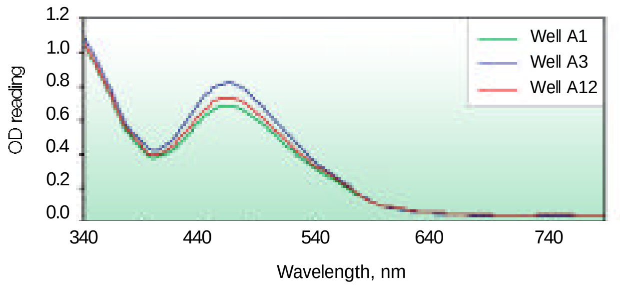

Fig. 1. Scan of sample wells with different cell densities across the wavelength range 340–800 nm. The absorbance of these samples showed a peak near 475 nm.

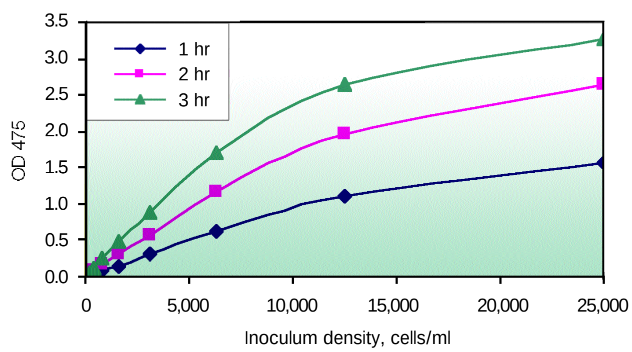

Fig. 2. Cell proliferation assay on A549 cells using XTT colorimetric detection and the Benchmark Plus microplate spectrophotometer.

Conclusion

The measurement of cell viability and cell division is used in a wide range of experiments to determine growth factor activity, for drug screening, and to determine the effects of chemical transfectants on human cells. We tested the Roche cell proliferation kit for measurement of cell density of A549 cells grown in 96-well plates. After 24 hr, when the cells were ~90% confluent in the wells with the most cells, we performed the cell proliferation assay.

At low cell density (cells ~50% confluent on the plate), there was a proportional increase in OD 475 with increasing cell number. At high cell density, the increase in OD 475 was not proportional to the increase in cell number. As seen in the figure, this increase was independent of the length of incubation with the reagent between 1 and 3 hr. However, at shorter incubation times (0.5 hr, data not shown), the OD 475 was proportional to cell number up to 50–70% confluence.

Bibliography

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