

Measuring Viability Decline in Animal Cell Suspension Cultures Using the Thermo Scientific NanoDrop 2000 Spectrophotometer.

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Introduction

Thermo Scientific™ NanoDrop™ spectrophotometers are the gold standard for the quantification and analysis of nucleic acids and proteins; however, there are a variety of other applications to which they are well suited.

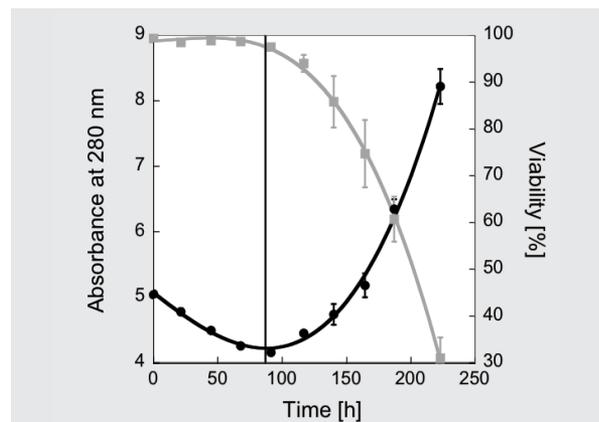
Growing cells (human, animal, or insect) in suspension is a common method used for the expression and purification of native or recombinant proteins. As such, monitoring cell growth is essential. The life cycle of cells grown in suspension can be divided into four distinct phases: lag phase, exponential growth phase, stationary phase, and decline or death phase. During the growth phase, nutrients in the cell culture media are being consumed expeditiously. These nutrients include, among other things, amino acids. In death phase, most of the nutrients have been consumed and cell growth slows down. Cell death (apoptosis) begins to occur causing the release of intracellular proteins into the growth media.

The accepted methods for determining the viability of any cell culture involve direct cell counting by visually inspecting cells through the use of dyes (such as trypan blue) or complicated microscopy techniques. These methods are invasive and time consuming. Applying the fact that cell death is directly correlated with an increase in free protein; quantifying this free protein is a simple way to measure the decline of cell viability (1). The Thermo Scientific NanoDrop 2000 Spectrophotometer is the ideal instrument for this assay.

Experimental Procedures

10-ml cell cultures were grown under standard conditions for Sf-9 (2) and mammalian cells CHO and HEK (3, 4). At 24 hour intervals two samples were removed from each culture. Cell viability of the first sample was determined using a traditional trypan blue assay. The second sample was centrifuged and the supernatant was measured using a NanoDrop 2000 Spectrophotometer. The resulting A_{280} measurements, over a 10 day period) were plotted over time.

Fig. 1 Average measured A_{280} values from cell culture supernatants (left axis, black dots) relative to average cell viability values (right axis, grey squares) of three cell lines grown in suspension, represented as function of cultivation time (x axis). R2 values of the curves are, respectively, 0.996 for A_{280} and 0.999 for viability.



Thermo Scientific NanoDrop 2000 Spectrophotometer

Results

A_{280} measurements correlated well with the phases of cell growth as shown in Fig. 1 (Values are the average of the three studied cell lines, for individual data refer to reference 1). This was confirmed using a traditional dye exclusion assay (Fig. 1). As expected, A_{280} values declined during exponential phase as UV-absorbing materials were consumed from the growth medium. As cells entered the death phase, A_{280} measurements began to increase as cell lysis progressed and UV-absorbing materials (i.e. proteins) were released into the medium.

Conclusions

This is an efficient method for monitoring cell growth phases, from lag through death phase. And because no dilutions are required, the method is less prone to sampling error, producing results that are more concordant compared to manual counting methods. In addition, because of the ease of use of the NanoDrop 2000 Spectrophotometer this method is simple and quick.

References

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