

Cell Proliferation and Viability Measurement

*Delicate Tasks...
...Require Precise Solutions*

Introduction

The quantification of cellular growth, including proliferation and viability, has become an essential tool in any laboratory working on cell-based studies. Such techniques enable not only the optimization of cell culture conditions, but also the determination of growth factor and cytokine activity. Even more importantly, the efficacy of therapeutic agents in drug screening, the cytostatic potential of anticancer compounds in toxicology testing, and cell-mediated toxicity can be assessed when quantifying cell growth.

The first step in any experiment is the decision whether to test for cell proliferation or viability parameters, depending on the objective of a study. These parameters are measured by assaying for “vital functions” that are characteristic for healthy or growing cells.

But how are the terms “cell proliferation” and “viability” defined?

Cell viability measurements assess healthy cells in a sample. This can be accomplished either by directly

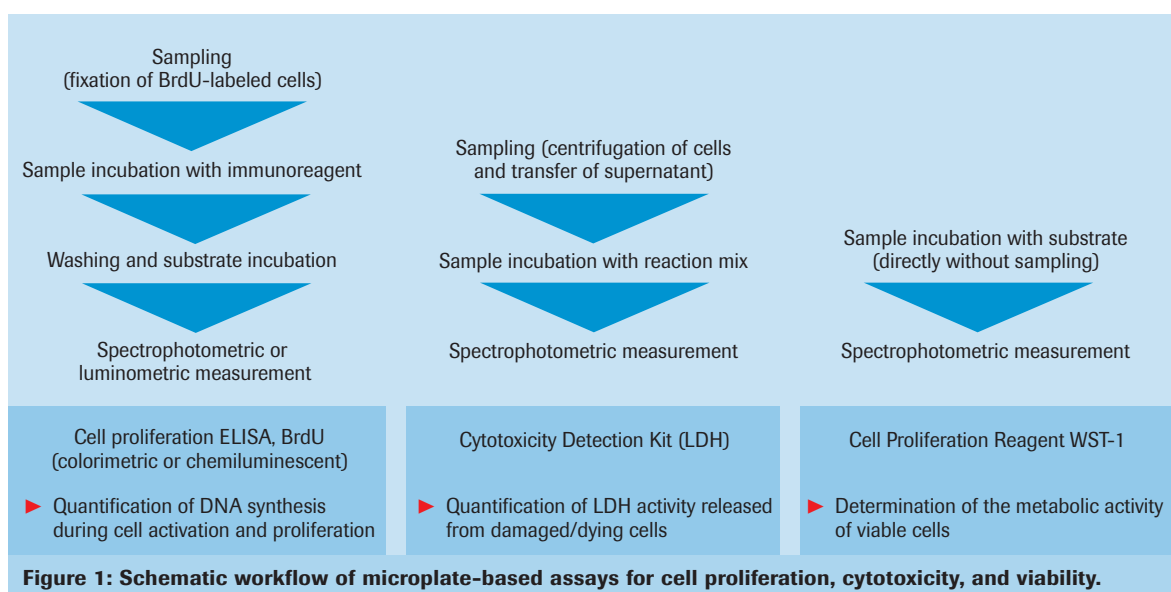
counting the number of healthy cells or by measuring an indicator for healthy cells in cell populations (e.g., in a microplate assay). Whether the cells are actively dividing or quiescent is not distinguished. An increase in cell viability indicates cell growth, while a decrease in viability can be interpreted as the result of either toxic effects of compounds/agents or suboptimal culture conditions.

In contrast to cell viability analysis, cell proliferation assessment is defined as the measurement of actively dividing cells in a sample. It can be expressed either as the actual number or proportion of proliferating cells in cell culture, tissues, or as relative values in assays for cell populations. Quiescent nongrowing healthy cells are not detected by cell proliferation assays.

Most Prominent Methods

Cell proliferation methods

The most prominent parameter for analyzing cell proliferation is the measurement of DNA synthesis as a specific marker for replication. In these assays, labeled DNA



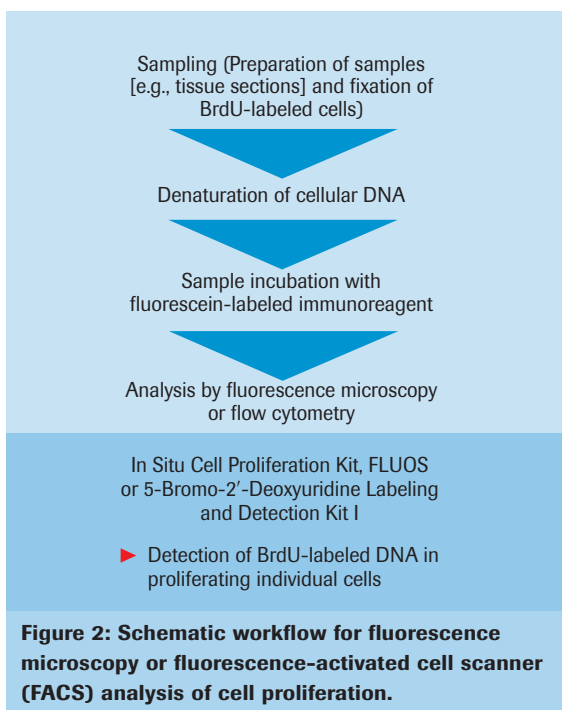
precursors ($[^3\text{H}]$ -Thymidine or 5-bromo-2'-deoxyuridine [BrdU]) are added to cells (or animals) and their incorporation into genomic DNA during the S phase of the cell cycle (replication) is quantified following incubation and sample preparation. The amount of labeled precursors incorporated into the DNA is quantified either by measuring the total amount of labeled DNA in a population, or by microscopically detecting the labeled nuclei. Incorporation of the labeled precursor into DNA is directly proportional to the rate of cell division occurring in the sample.

Particularly when studying nonsynchronized cell cultures with cells in different phases of the cell cycle, it is important to choose a suitable labeling period. Results will vary strongly depending not only on the growth rate, but also on whether the incubation with labeled DNA precursors is performed for a short period or for a complete division cycle. This is important to keep in mind for the interpretation of the results of such an assay.

Cell proliferation can also be measured using indirect parameters. With these techniques, molecules that regulate the cell cycle are quantified either by measuring their activity (e.g., cyclin-dependent kinase assays) or by direct quantification (e.g., Western blots, ELISA, or immunohistochemistry).

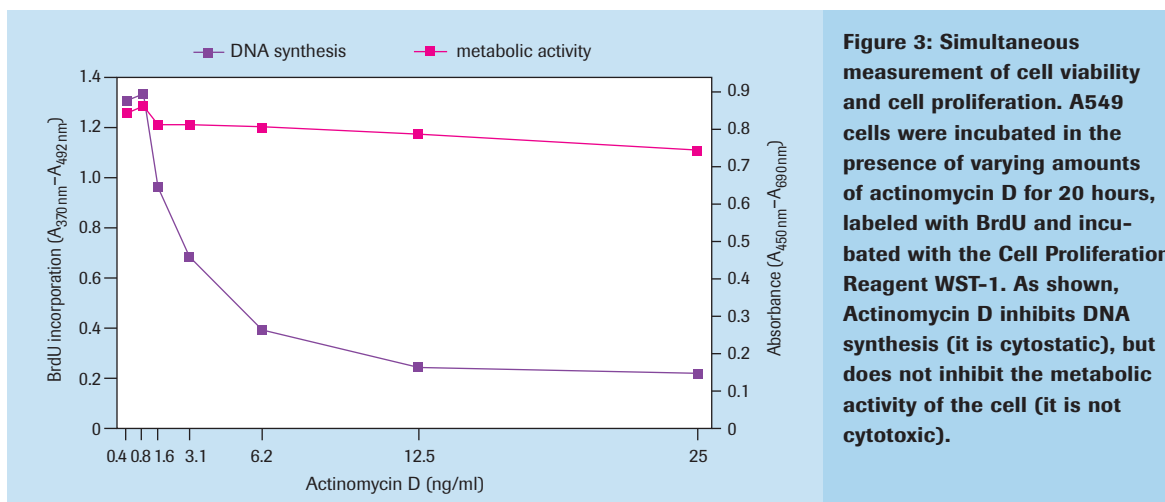
Cell viability methods

Most viability assays are based on one of two characteristic parameters: metabolic activity or cell membrane integrity of healthy cells. Usually the metabolic activity is measured in cell populations via incubation with a tetrazolium salt (e.g., MTT, XTT, WST-1) that is cleaved into a colored formazan product by metabolically active cells.



The ATP status of cells can also be analyzed and gives an indication for cellular energy capacity and thus viability.

The second type of assay – also termed dye-exclusion assay – takes advantage of the ability of healthy cells with uncompromised cytoplasmic membrane integrity to exclude dyes such as trypan blue. Dead cells will be stained, and healthy cells can be counted directly. In cytotoxicity studies, this ability is also analyzed; however, in these assays, molecules are released from dying cells with leaky cytoplasmic membranes, and are measured in cell populations (e.g., lactate dehydrogenase or $[^51\text{Cr}]$ release).



Assay Procedures for Cell Proliferation and Viability Kits

Roche Applied Science offers a broad range of reliable and easy-to-use products for the analysis of cell proliferation and viability. Selected examples are given in Figures 1 and 2. The assay procedures for microplate tests, which are particularly useful for routine applications in high-volume labs, are summarized in Figure 1. Figure 2 shows the workflow for an application on individual cells.

Simultaneous measurement of cell proliferation and cell viability

A549 cells (1×10^4 /well in 100 μ l) were incubated in the presence of varying amounts of actinomycin D for 20 hours. After labeling of the cells with BrdU for 2 hours, Cell Proliferation Reagent WST-1 was added, and the cells were reincubated for another 2 hours. Then, the formazan product was quantified at 450 nm with an absorbance reader (Figure 3). Subsequently, BrdU incorporation was determined using the Cell Proliferation ELISA, BrdU (colorimetric). The results show that Actinomycin D inhibits DNA synthesis, but it does not inhibit the metabolic activity of the cell. Thus, actinomycin D is cytostatic (inhibits DNA synthesis) but not cytotoxic (no inhibition of metabolic activity).

For more information, visit our new apoptosis and cell-proliferation special-interest site at www.roche-applied-science.com/apoptosis



Product	Pack Size	Cat. No.
BrdU Labeling and Detection Kit I	1 kit (100 tests)	1 296 736
BrdU Labeling and Detection Kit II	1 kit (100 tests)	1 299 964
BrdU Labeling and Detection Kit III	1 kit (1,000 tests)	1 444 611
In Situ Cell Proliferation Kit, FLUOS	1 kit (100 tests)	1 810 740
Cell Proliferation ELISA, BrdU (colorimetric)	1 kit (1,000 tests)	1 647 229
Cell Proliferation ELISA, BrdU (chemiluminescent)	1 kit (1,000 tests)	1 669 915
Anti-BrdU, Formalin Grade	50 μ g (500 μ l)	1 170 376
Anti-BrdU-Fluorescein, Formalin Grade	50 μ g (500 μ l)	1 202 693
Anti-BrdU-Peroxidase, Fab fragments, Formalin Grade	15 U	1 585 860
Cell Proliferation Kit I (MTT)	1 kit (2,500 tests)	1 465 007
Cell Proliferation Kit II (XTT)	1 kit (2,500 tests)	1 465 015
Cell Proliferation Reagent WST-1	1 kit (2,500 tests)	1 644 807