2.2 Methods for studying cell proliferation and viability in individual cells

As mentioned in Section B 1, the viability as well as proliferation of individual cells can be assessed by standard microscopic methods. For instance, cells may be treated with a vital stain or exclusion dye and counted directly in a hemocytometer. The same cell parameters may be determined by flow cytometry if the cells are differentially stained with fluorescent dyes that bind DNA (DNA fluorochromes), see also section A 2.2.3 on page 49 of this guide.

In the following section we will describe details of the following proliferation assays:

- Assays that measure DNA synthesis: As outlined above, if labeled DNA precursors are added to the cell culture, cells that are about to divide incorporate this precursor into their DNA (described on the following pages of this guide)

2.2.1 Assays that measure DNA synthesis

Studies of cell proliferation *in vivo* as well as on individual cells *in vitro* frequently employ [^{3}H]-TdR to label the DNA of replicating cells and autoradiography to reveal the radioactive label. As a nonradioactive alternative, bromodeoxyuridine (BrdU) can be used to label proliferating cells *in vivo* and *in vitro*. Incorporated BrdU can be detected by immunohistochemistry, immunocytochemistry or flow cytometry.

Immunochemical techniques allow both the visualization of dividing cells and the detection of tissue morphology by counterstaining (*e.g.,* with hematoxylin and/or eosin). Thus, it is possible to visualize cells which have incorporated BrdU into DNA in its natural environment and to localize cell position in the tissue[73, 74].

As only those cells which are actually in the S-phase (DNA-synthesis) of the cell cycle will be labeled, the so-called “labeling index” can be determined if the labeled nucleotide ([^{3}H]-TdR or BrdU) is present for only short periods of time (*e.g.,* 15–60 minutes). The “labeling index” (proportion of S-phase cells in an asynchronously growing population) is calculated by dividing the number of labeled cells by the total number of cells in the entire population.

While short labeling periods (pulse labeling) are suitable to quantify the percentage of S-phase cells within a cellular population, longer labeling periods (*e.g.,* for a whole cell cycle transition) can be used to determine a replicating population.

Roche Applied Science offers several kits and reagents for measuring proliferating cells by BrdU incorporation. These products are described on the following pages.

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