

2. Cell proliferation/viability assay methods

A variety of methods have been devised that measure the viability or proliferation of cells *in vitro* and *in vivo*. These can be subdivided into four groups:

- ① Reproductive assays can be used to determine the number of cells in a culture that are capable of forming colonies *in vitro*. In these types of experiments, cells are plated at low densities and the number of colonies is scored after a growth period. These clonogenic assays are the most reliable methods for assessing viable cell number^{57, 58, 59}. These methods, however, are very time-consuming and become impractical when many samples have to be analyzed.
- ② Permeability assays involve staining damaged (leaky) cells with a dye and counting viable cells that exclude the dye. Counts can either be performed manually using a hemocytometer and for example trypan blue (Figure 52). This method is quick, inexpensive, and requires only a small fraction of total cells from a cell population. Therefore, this method is generally used to determine the cell concentration (cell number/ml) in batch cell cultures. This is helpful in ensuring that cell cultures have reached the optimal level of growth and cell density before routine sub-culture, freezing, or any experiment⁶⁰.

Or counts can be performed mechanically using for example a flow cytometer and propidium iodide. Alternatively, membrane integrity can be assayed by quantifying the release of substances from cells when membrane integrity is lost, *e. g.*, Lactate dehydrogenase (LDH) or ⁵¹Cr (described in section A 3.2.1 starting on page 59 of this guide).

- ③ Metabolic activity can be measured by adding tetrazolium salts to cells. These salts are converted by viable cells to colored formazan dyes that are measured spectrophotometrically (described in section B 2.1.1 starting on page 82 of this guide).
- ④ Direct proliferation assays use DNA synthesis as an indicator of cell growth. These assays are performed using either radioactive or nonradioactive nucleotide analogs. Their incorporation into DNA is then measured (nonradioactive assays are described in section B 2.1.2 starting on page 89 of this guide).



▲ Figure 52: Measurement of proliferation by counting the cells with a hemocytometer. The addition of trypan blue helps to distinguish viable, unstained cells (○) from non-viable, blue-stained cells (●).

The first section describes those assays designed to study cell proliferation in whole populations of cells, followed by a section covering proliferation assays designed to measure proliferation in individual cells (*in situ*).

For a discussion of the advantages and limitations of all types of cell proliferation assays, read Sections B 2.1.3 and B 2.2.2 of this guide.

For discussions of particular assays, turn to the pages indicated in the following method selection guide.

B