

3.2 Methods for studying cytotoxicity

Most current assays for measuring cytotoxicity are based on alterations of plasma membrane permeability and the consequent release (leakage) of components into the supernatant or the uptake of dyes, normally excluded by viable cells (Figure 41) (see also A 2.2.3, on page 49 “Dye exclusion method”). A serious disadvantage of such permeability assays is that the initial sites of damage of many, if not most cytotoxic agents are intracellular. Therefore, cells may be irreversibly damaged and committed to die and the plasma membrane is still intact. Thus, these assays tend to underestimate cellular damage when compared to other methods. Despite this fact, some permeability assays have been widely accepted for the measurement of cytotoxicity.

Alternatively, dead cells are unable to metabolize various tetrazolium salts (see also Section A 2.1.1). This allows the use of the colorimetric assays MTT, XTT, or WST-1 to measure cell survival. Apoptosis, however, is an active mode of cell death requiring the metabolism of cells. Thus, like the permeability assays mentioned above, the colorimetric assays may underestimate cellular damage and detect cell death only at the later stages of apoptosis when the metabolic activity of the cells is reduced.

Regardless of this disadvantage, the colorimetric assays are very useful for quantitating factor-induced cytotoxicity within a 24 to 96 h period of cell culture. However, these colorimetric assays are of limited value for measuring cell mediated cytotoxicity, since most effector cells become activated upon binding to the target cells. This activation results in an increased formazan production by the effector cell, which tends to mask the decreased formazan production resulting from target cell death.

Note: Assays for cytotoxicity can be, and frequently are, used to measure cell necrosis.

3.2.1 Assays that measure plasma membrane leakage

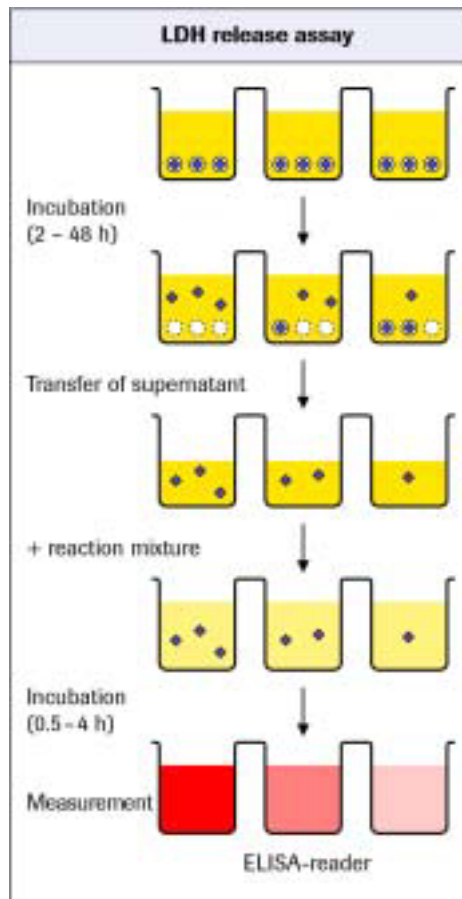
Widely used standard methods for measuring plasma membrane leakage are based on the uptake or exclusion of molecules with special light-absorbing or fluorescent properties. Viable (intact plasma membrane) and dead (damaged plasma membrane) cells can be discriminated by differential staining. Because dyes stain individual cells, each sample has to be analyzed by flow cytometry or microscopy. This kind of single cell analysis is not suitable if many different samples have to be measured. In contrast, assays which quantitate plasma membrane disintegration in cell populations allow many different samples to be handled simultaneously in a single MTP.

One group of standard assays performed in a MTP is based on the release of radioactive isotopes ($[^{51}\text{Cr}]$, $[^3\text{H}]$ -thymidine, $[^3\text{H}]$ -proline, $[^{35}\text{S}]$ - or $[^{75}\text{Se}]$ -methionine, 5- $[^{125}\text{I}]$ -2-deoxy-uridine) or fluorescent dyes (bis-carboxyethyl-carboxyfluorescein (BCECF) or calcein-AM) from prelabeled target cells^{48, 49, 50}. The disadvantages of such assays however, are (i) the use of radioactive isotopes in most of them, (ii) the necessity for prelabeling of the target cells, and (iii) the high spontaneous release of most labels from the prelabeled target cells.

Another group of assays is based on the measurement of cytoplasmic enzymes released by damaged cells. The amount of enzyme activity detected in the culture supernatant corresponds to the proportion of lysed cells^{51, 52}. Enzyme release assays have been described for alkaline and acid phosphatase, for glutamate-oxalacetate transaminase, for glutamate pyruvate transaminase, and for argininosuccinate lyase. However, their use has been hampered by the low amount of those enzymes present in many cells and by the elaborate kinetic assays required to quantitate most enzyme activities.



In contrast to the above mentioned cytoplasmic enzymes, lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme present in all cells. It is rapidly released into the cell culture supernatant when the plasma membrane is damaged. With the Cytotoxicity Detection Kit (see page 61), LDH activity can easily be measured in culture supernatants by a single point assay. The use of a spectrophotometric microplate reader (ELISA plate reader) allows the simultaneous measurement of multiple probes and thereby guarantees the easy processing of a large number of samples (Figure 42).



▲ Figure 42: Measurement of LDH activity (◆) using the microplate format (see also Flow Chart 12).