2.1 Methods for studying cell proliferation and viability in cell populations

A number of methods have been developed to study cell viability and proliferation in cell populations. The most convenient modern assays have been developed in a microplate format (96-well plates). This miniaturization allows many samples to be analyzed rapidly and simultaneously. The microplate format also reduces the amount of culture medium and cells required as well as cost of plasticware. Colorimetric assays allow samples to be measured directly in the microplate with an ELISA plate reader.

Microplate assays have been developed based on different parameters associated with cell viability and cell proliferation. The most important parameters used are metabolic activity and DNA synthesis for microplate format.

- Cellular damage will inevitably result in loss of the ability of the cell to maintain and provide energy for metabolic cell function and growth. Metabolic activity assays are based on this premise. Usually they measure mitochondrial activity. The cells are incubated with a colorometric substrate (MTT, XTT, WST-1) (described on pages 82-87 of this guide.
- As outlined above, during the S phase the cell undergoes DNA synthesis and replicates its genome. If labeled DNA precursors, in our case BrdU, are added to the cell culture, cells that are about to divide incorporate BrdU into their DNA. The incorporated BrdU can then be detected by a quantitative cellular enzyme immunoassay using monoclonal antibodies against BrdU (described on pages 88-107 of this guide).

In the following sections we will describe details of each of these cell viability and proliferation assays.

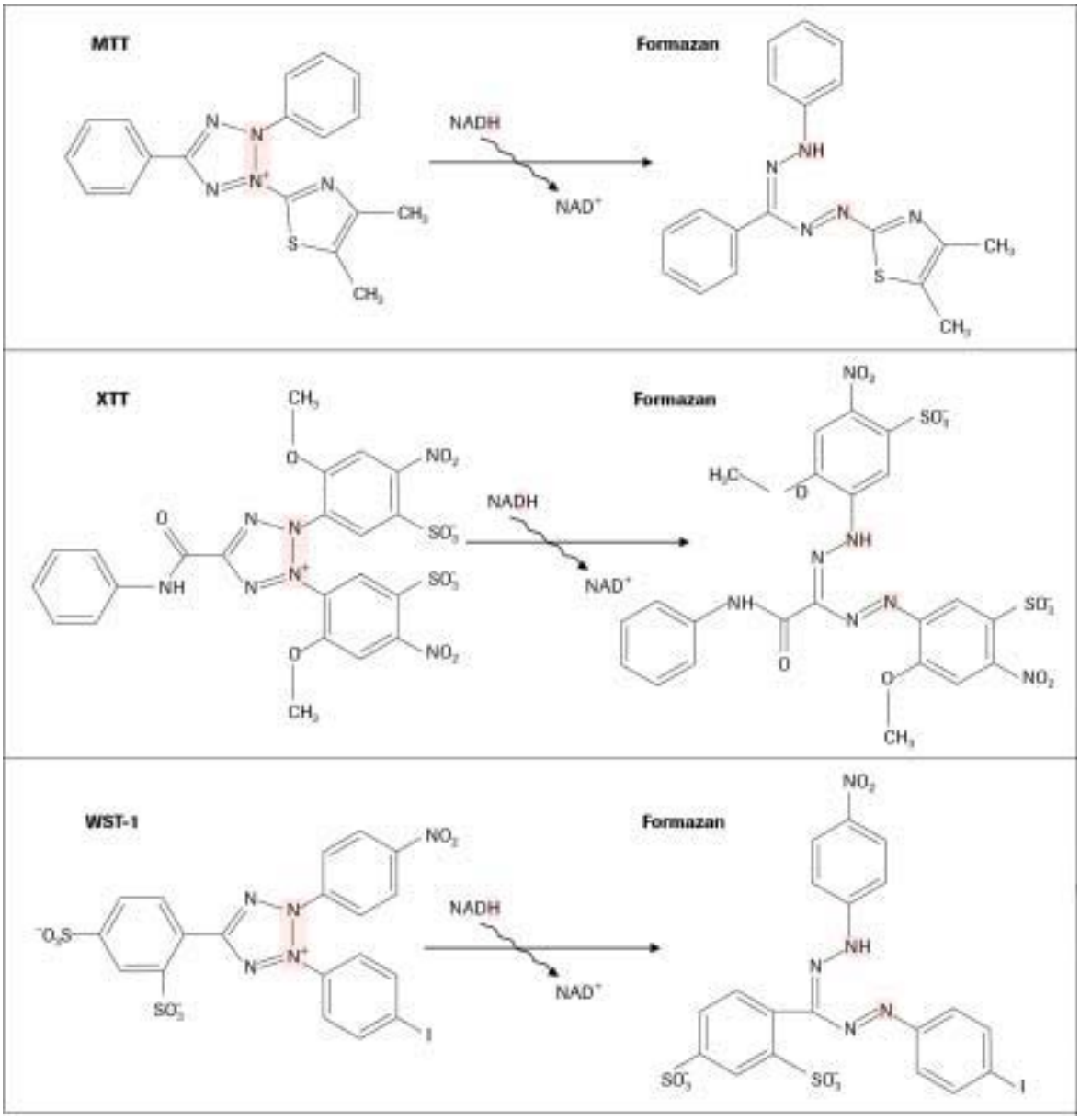
2.1.1 Assays that measure metabolic activity

One parameter used as the basis for colorimetric assays is the metabolic activity of viable cells. For example, a microtiter plate assay which uses the tetrazolium salt MTT is now widely used to quantitate cell proliferation and cytotoxicity[53, 65].

Because tetrazolium salts are reduced to a colored formazan only by metabolically active cells, these assays detect viable cells exclusively. For instance, in the MTT assay, MTT is reduced by viable cells to a colored, water-insoluble formazan salt. After it is solubilized, the formazan formed can easily and rapidly be quantitated in a conventional ELISA plate reader at 570 nm (maximum absorbance).

[Author's note: MTT is cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the mitochondrial respiratory chain and is active only in viable cells. Interestingly however, recent evidence suggests that mitochondrial electron transport may play a minor role in the cellular reduction of MTT. Since most cellular reduction occurs in the cytoplasm and probably involves the pyridine nucleotide cofactors NADH and NADPH, the MTT assay can no longer be considered strictly a mitochondrial assay.]

More recently, modified tetrazolium salts like XTT[62, 67], MTT[68], and WST-1 (Figure 54) have become available. The major advantage of these compounds is that viable cells convert them to a water-soluble formazan. Thus, a metabolic assay with any of these compounds requires one less step (solubilization of product) than an assay with MTT. In addition, WST-1 is stable enough to be packaged as a ready-to-use solution.



▲ **Figure 54: Molecular structure of MTT, XTT, WST-1 and their corresponding reaction products.**

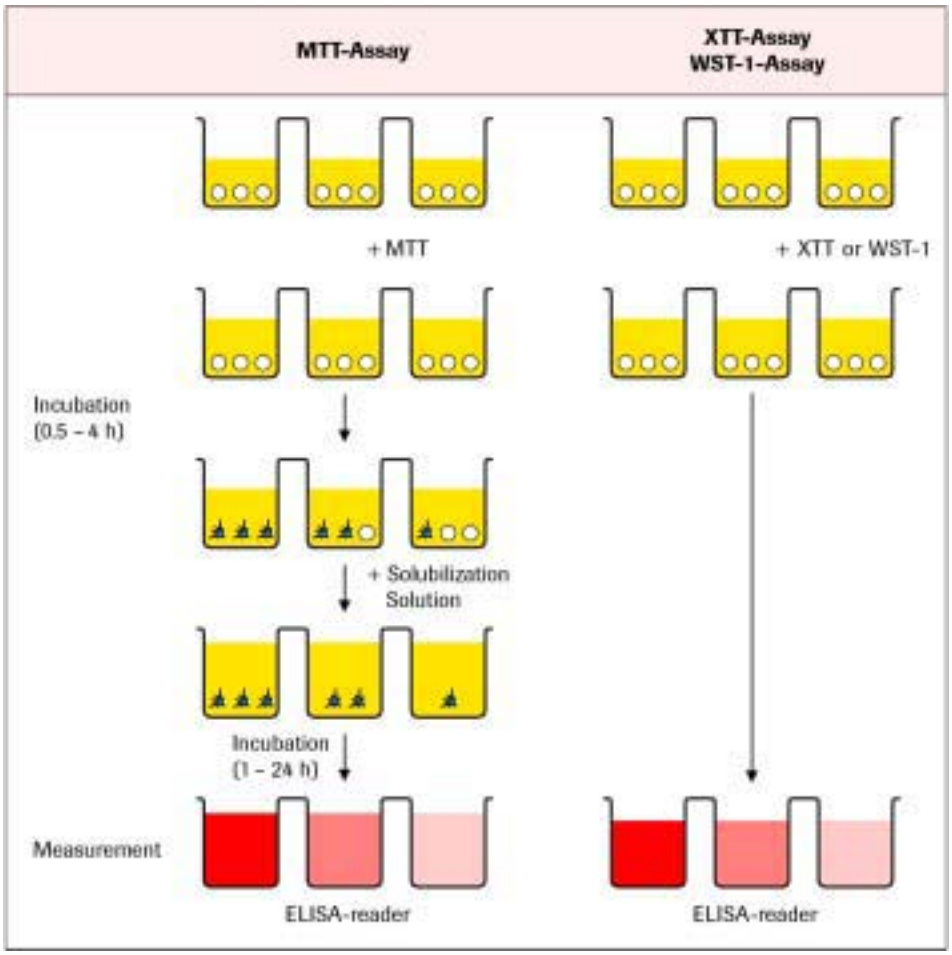
Since proliferating cells are metabolically more active than non-proliferating (resting) cells, the assays are suitable not only for the determination of cell viability and factor-mediated cytotoxicity (see Section A 3.2.2.) but also for the determination of cell activation and proliferation. However, one has to keep in mind that under non-ideal cell culture conditions (such as the pH and D-glucose concentration in culture medium), the MTT response may vary greatly in viable cells due to the metabolic state of the cells (*e.g.*, cellular concentration of pyridine nucleotides)[65, 69].

These colorimetric assays are very rapid and convenient. Because this technique needs no washing or harvesting of the cells, the complete assay from the start of the microculture to data analysis in an ELISA plate reader is performed in the same microplate. In addition, an ELISA plate reader linked with a computer allows rapid and automated data processing (Figure 55).

Roche Applied Science offers three microplate-based assays similar to the ones described in this section. All three assays are suitable for measurement of cell proliferation in response to growth factors, cytokines, mitogens and nutrients.

One of these assays uses MTT, which forms an insoluble formazan product; the other two use tetrazolium salts (XTT and WST-1) that form soluble formazan products. All three assays are described on the following pages.

Note: For a more detailed discussion of the principles behind these metabolic assays, see the topic, "Biochemical and cellular basis of cell proliferation assays that use tetrazolium salts" (Appendix, page 121) in this guide.



▲ **Figure 55: Measurement of metabolic activity using the tetrazolium salts MTT, XTT and WST-1.**