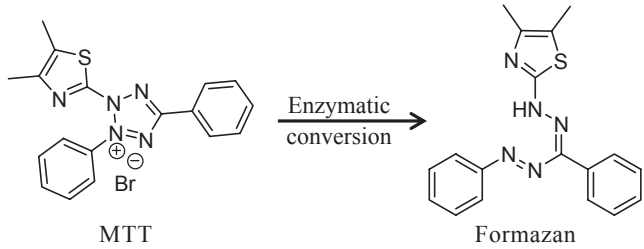

FIGURE 8.8 The MTT human hepatotoxicity assay monitors the rate of conversion of MTT to formazan in a cell culture. Compounds that are cytotoxic decrease the rate of production of formazan from MTT.

purple compound (Figure 8.8). This reaction occurs in the mitochondria of cells at a known rate, and concentrations of both MTT and formazan can be measured spectroscopically. Cytotoxic compound will decrease the rate of conversion, making it possible to screen for this potential safety issue. In addition, since this assay uses hepatocytes (e.g., liver cells), compounds that are converted to cytotoxic metabolites will also be identified in this assay.[20]

The lactate dehydrogenase (LDH) assay[21] and the neutral red assay[22] are also popular methods of identifying compounds that represent a cytotoxicity risk. Both of these assays can be run with hepatocytes, preserving the ability to identify compounds that produce cytotoxic metabolites in the liver. In the LDH assay, the amount of LDH (Figure 8.9(a)) released by dead cells after an incubation period with candidate compounds is used as an indicator of cytotoxicity. Quantitation of the production of formazan by LDH can be accomplished using commercially available kits (Roche, catalog number 11644793001). Since LDH is not released by living cells, increased concentration of LDH in the cell growth media is an indication of cytotoxicity. In a similar manner, healthy hepatocytes absorb the dye neutral red (Figure 8.9(b)) and sequester it in lysosomes.

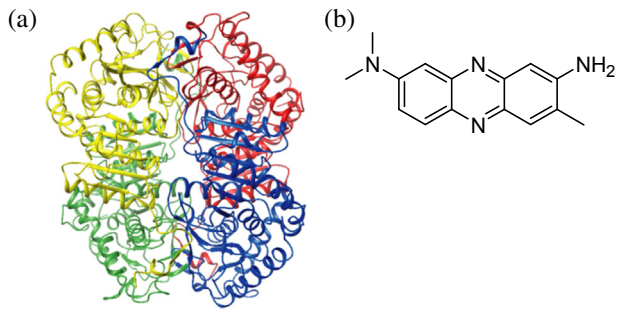

FIGURE 8.9 (a) Lactate dehydrogenase (LDH) (RCSB PDB file 1T2F). (b) Neutral red.