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Defining optimal MTT assay conditions in SH-SY5Y cells: A rotenone-based *in vitro* model of Parkinson's disease

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Although the MTT assay is widely used to evaluate cell viability and cytotoxicity, its results are highly dependent on experimental parameters such as cell density, reagent concentration, and incubation conditions. In neuronal models, particularly in SH-SY5Y cells used for Parkinson's disease studies, a lack of standardized optimization may lead to variability and misinterpretation of results. Therefore, the present study aimed to systematically optimize key parameters of the MTT assay and evaluate their impact under both normal and neurotoxic conditions. To optimize the MTT assay in SH-SY5Y cells, we evaluated the effects of cell number, MTT concentration, incubation time, post-DMSO incubation period, and optical density at different wavelengths. These parameters were further examined in a rotenone-induced *in vitro* Parkinson's disease model. In addition, results obtained from DMSO-treated SH-SY5Y cells were compared under varying assay conditions. Our results indicated that while the MTT method is affected by many parameters, the cell number, MTT concentration, measurement wavelength, and the waiting time after dissolving formazan crystals with DMSO have significant effects on OD values. Consequently, we determined that optimizing this method, which determines cell viability, toxicity, or cell metabolism, is crucial for obtaining and interpreting rational results.

Keywords: Neuroblastoma cells, assay optimization, metabolic activity, dopaminergic neuron model