

Comparison of Visual Inspection to CellTiter-Glo[®] in Evaluating Cytotoxicity in the LUMI-CELL[®] ER Bioassay

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There is a need to determine if a decreased response observed (for agonist and in particular antagonist testing) in an endocrine disruptor screening assay is a result of lower activity due to a chemical-receptor response or whether the compound is cytotoxic, resulting in a lower observed response. A comparison study was conducted in cooperation with NICEATM to compare cytotoxicity measured using the Visual Inspection assay with that obtained using Promega's CellTiter-Glo[®] assay which is a method of estimating viable cell number based on quantitation of ATP. Visual Inspection viability score codes were developed with: 1 = normal cell morphology and cell density, 2 = altered cell morphology and small gaps between cells, 3 = altered cell morphology and large gaps between cells and, 4 = few (or no) visible cells. Comparison of the Visual Inspection and CellTiter-Glo[®] assays demonstrate that, a score of 1 corresponded to greater than 80% viability, 2 corresponded to 80 – 60% viability, 3 corresponded to 60 – 40% viability and 4 corresponded to less than 40% viability. Eight coded compounds were selected to test for estrogenic activities and eight coded substances to test antagonist activities in the LUMI-CELL[®] ER assay, a cell based assay in which estrogenic chemicals induce firefly luciferase. The CellTiter-Glo[®] and Visual Inspection assays both were able to detect cytotoxicity, with a significant correlation between the two techniques for determining cytotoxicity. Accordingly, either of these methods may be useful in determining cytotoxicity of chemicals and extracts tested in the LUMI-CELL[®] ER assay, thereby eliminating cytotoxicity-dependent false positives. Supported by NIEHS Contract N01-ES-85424, NIEHS SBIR ES10533-03 and Superfund Basic Research Grant ES04699.