Performance of the BG1Luc and ER β-Lactamase Estrogen Receptor Transactivation Assays in Tox21

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Two estrogen receptor (ER) transactivation (TA) assays, the BG1Luc and HEK293 ER β-lactamase (ER-bla) methods, were adapted for use in the U.S. Tox21 high-throughput screening program. Both *in vitro* assays detect substances with ER agonist (Ag) or Antagonist (Ant) activity. BG1Luc endogenously expresses full-length ER (α and β) and is stably transfected with a plasmid containing four estrogen responsive elements (ERE) upstream of a luciferase reporter gene. ER-bla is a mammalian one-hybrid system stably expressing a β-lactamase reporter gene under the control of the GAL4 DNA-binding site and a fusion protein consisting of the human ERα ligand-binding domain and the GAL4 DNA-binding domain. Approximately 10,000 chemicals were tested three times in both assays in Ag and Ant modes. To differentiate true ER Ants from cytotoxic substances, cell viability was determined. Concentration-response data (N=15) were analyzed to evaluate the performance of the two assays. The assay data quality was high in both Ag and Ant modes as indicated by acceptable signal to background ratio (2.5 to 8), CV (<10.5%), reproducibility (outcome matches across triplicate runs, ≥87%), and Z' factor (≥0.4). Sensitivity and specificity of the assays were compared to ER TA performance standards that were developed by ICCVAM for the BG1 manual method. . Ag accuracy was 97% for BG1, 90% for Bla; ant accuracy was 100% for both assays. Ag sensitivity was 96% for BG1, 87% for ER-Bla, and 100% for both Ant assays. Specificity was 100% for BG1 and ER-Bla in both modes. Reference standard values were: estradiol EC_{50} 30 pM for BG1 and 275 pM for ER-Bla, and hydroxytamoxifen IC_{50} 71 nM for BG1 and 6 nM for ER-Bla. Understanding the differences behind the performance of these assays is critical to their acceptance and utilization by both regulators and industry.

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