

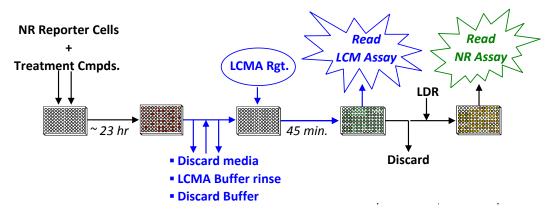
## Live Cell Multiplex (LCM) Assay

Ewa Maddox and Bruce Sherf, INDIGO Biosciences, Inc., State College, PA

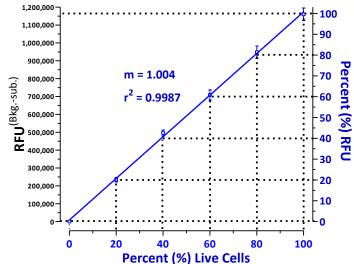
The Live Cell Multiplex (LCM) Assay provides an efficient fluorescence-based method of quantifying LIVE cells resident in treated wells of an assay plate. The assay chemistry is specifically optimized to be run in combination with any of INDIGO's 96-well format Nuclear Receptor Reporter System Assay products.

The LCM Assay allows users to validate their Nuclear Receptor Assay data by determining if their treatment compounds exert mitogenic, cytostatic or cytotoxic activities on the reporter cells. The occurrence of such adverse non-specific effects will always undermine the accurate assessment of a test compound's potency and/or efficacy as a modulator of nuclear receptor function.

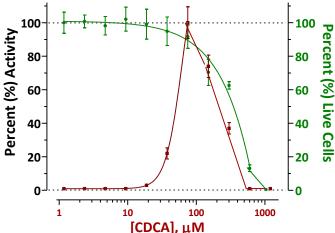
In particular, when screening test compounds for *antagonist* activities, an observed drop in luciferase activity may be incorrectly attributed to specific inhibition of the nuclear receptor. In reality, however, the treatment condition may have pushed the reporter cells into division arrest, apoptosis, necrosis, or lysis. The INDIGO Live Cell Multiplex Assay provides a sensitive, convenient method of identifying such cytotoxic responses, and thus eliminating 'false-positive' data.



**INDIGO's Live Cell Multiplex and Nuclear Receptor Assays.** Measurements are performed sequentially from the same assay wells.



% RFU = % Live Cells. The LCM Assay provides a direct correlation between % RFU and % Live Cells per well.



**FXR -LCM Assay.** Agonist CDCA exhibits an upper threshold concentration, above which FXR activity plummets. The LCM Assay reveals the decline in FXR activity to be the result of CDCA-induced cell death.