

High-throughput assay for cell viability based on calcein staining

HNMPA (hydroxy-2-naphthylmethyl)-phosphonic acid), an insulin receptor tyrosine kinase inhibitor, is used in this assay as a modulator in order to identify small-molecule suppressors of its action. In this way, we hope to identify small-molecular activators of the insulin signaling pathway. HNMPA causes cell death of HepG2 cells in a dose-dependent manner (Wagner, unpublished results), so we use the viability dye calcein-AM (Molecular Probes) to detect any compound-induced increase in cell number.

1. 2000 HepG2 hepatoma cells in media containing 0.5% FBS and 10 μ M HNMPA are seeded per well of a black 384-well plate, using a Precision 2000 automated plate filler (BioTek Instruments, Inc., Winooski, VT).
2. Immediately following seeding, compounds, stored one per well in DMSO in 384-well plates, are transferred to each well. Approximately 100nL is transferred by an Assay TekBench robotic liquid handler (TekCel, Inc., Hopkinton, MA).
3. Twenty-four hours later, the media is aspirated, and 50 μ L of 1 μ M calcein-AM, dissolved in PBS, is added to each well. The plates are incubated at room temperature for 1 hour.
4. Following incubation, the cells are washed once with PBS, and fluorescence is measured with an Analyst AD automated plate reader (Molecular Devices, Sunnyvale, CA) using a 480nm excitation filter and a 530nm emission filter.