

High-throughput phenotypic assay for adipocyte differentiation

Collaboration with Y. Tseng, C.R. Kahn

1. Immortalized brown preadipocytes are cultured according to Tseng *et al.* (2004). Upon reaching appropriate density in T175 flasks, cells are seeded into clear-bottomed, or “optical,” 96-well plates (100 μ L/well, 10000 cells/well) or optical 384-well plates (50 μ L/well, 5000 cells/well) in differentiation media (DM). The 384-well plates are used for high-throughput screening with compounds, while the 96-well plates are used for lower throughput follow-up.
2. Forty-eight hours after seeding the plates (day 0), the media is aspirated with a ELx405 multichannel aspirator (BioTek Instruments, Inc., Winooski, VT) and replaced with fresh DM with a Precision2000 automated plate filler (also BioTek, Instruments, Inc.).
3. Compounds, stored one per well in DMSO in 384-well plates, are pin-transferred to each well of the assay plate. Approximately 100nL is transferred by an Assay TekBench robotic liquid handler (TekCel, Inc., Hopkinton, MA).
4. Forty-eight hours after compound treatment (day 2), the media is again aspirated and replaced with fresh DM.
5. Forty-eight hours later (day 4), the media is aspirated and the cells stained with oil red O for lipid accumulation. The protocol of Tseng *et al.* (2004) is followed for staining.
6. After staining, the wells are air-dried. Following drying, 4% NP-40 in PBS is added to each well, and the plates incubated for 15 minutes, to extract incorporated dye. This step is included to create an evenly stained appearance in each well, facilitating automated reading with a plate reader.
7. Absorbance is measured with an Analyst AD automated plate reader (Molecular Devices, Sunnyvale, CA) using a 530nm filter.

Reference:

Tseng YH, Kriauciunas KM, Kokkotou E, Kahn CR. (2004) Differential roles of insulin receptor substrates in brown adipocyte differentiation. *Mol Cell Biol* 24:1918.