**Protocol for Primary Human Hepatocytes Metabolism Assays**

1. Prepare chemicals for dosing at 2X the desired concentration (20 M) in Williams E Media and transfer 50 µL aliquots to the assay plates. Will need a minimum of 1.6 mL.
2. Thaw 8 vials of PHHs (50 donor pooled) *LiverPool 50 Donor Lot: HZD* and transfer as quickly as possible to hepatocyte thawing media (only thaw max of 2 vials per tube of thawing media).
3. Rinse vials of PHHs with ~ 1 mL of thawing media to be sure to get all cells from vial.
4. Centrifuge PHH suspensions at ~100xg for 10 min
5. Remove thawing media from conical tube being careful not to disturb the cell pellet.
6. Resuspend PHHs in ~5 mL of room temperature buffered WEM (15mM HEPES).
7. Combine all 4 tubes of cells into a single tube.
8. Count viable and total cell numbers using the Cellometer and adjust viable cell density to a final concentration of ~50,000 cells/50µL (~1.0x106 cells/mL) in buffered WEM with 15 mM HEPES). We will need at least 40 mL.
9. Transfer cell suspensions to a trough and using a multichannel pipettor transfer 50µL of diluted PHH cell suspensions to the assay plate to start metabolism reactions as shown in the plate map (do not add cells to the no-cell control wells).
10. Move plates to 37oC Incubator at ~5% CO2 and incubate dependent on time points.
11. At time points (15, 30, 60, 90, 120, and 240 minutes) add 100µL of acetonitrile to the assay wells.
12. Spin down 96 well plates