Icahn School of Medicine at Mount Sinai LINCS Center for Drug Toxicity Signatures

Standard Operating Procedure:
Drug Treatment and Cell Lysis for PromoCell Cardiomyocytes

DToxS SOP Index: CE-4.0
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Approvals (Date):		
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Quality Assurance/Control (QA/QC) steps are indicated with green highlight.

Metadata recording is highlighted with yellow highlight and superscript indices.

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1. Calculate the dilution factor for the drug of interest and thaw the master stock of drugs as prepared by DToxS SOP CE-3.0
    a. We store master stocks for all drugs in the 10 mM range. The final concentration of each drug in the treatment medium is determined based on its corresponding plasma levels in humans; therefore, the dilution factor varies for each drug.
    b. If the dilution factor is greater than 1:4,000, it is recommended to prepare an intermediate working stock. For example, for a 1:10,000 dilution, we recommend two serial 1:100 dilutions.
    c. All dilutions should be prepared aseptically
2. Take the cells out of the incubator and aseptically add the appropriate amount of drug
3. Culture cells for an additional 48 hours at 37ºC / 5% CO_2 without any media changes
4. After 48 hours of drug treatment, place the 60 mm culture dishes (Corning, Cat: 430166) on ice, rapidly aspirate media, and gently wash twice with 5 mL of 4°C 1XPhosphate Buffered Saline (1XPBS) in each wash
    a. Protocol volumes can be scaled by dish/plate surface area proportionally.
5. Lyse cells
    a. Aspirate PBS completely and move everything under a chemical hood
    b. Add 2 mL Trizol under a chemical hood
    c. Wait for 5 minutes
6. Collect the cell lysate
    a. Scrape the cells off the dish with a sterile cell scraper (Fisher, Cat: 08-100-241)
    b. Pipette up and down several times with a 1000 µL micropipette

c. Transfer 1 mL of lysate into two 1.5 mL tubes. For this and all subsequent steps, use Protein LoBind tubes (Eppendorf, Cat: 022431081), and keep the two solutions separated until the last steps of assay preparation as noted in DToxS SOPs A-1.0 and A-2.0.

7. Freeze lysate at -80ºC or continue with RNA and/or protein isolation protocols (DToxS SOPs A-1.0 and A-2.0)