Making cell line samples for proteomic analysis

Purpose:

This protocol describes how to make cell line lysates for downstream proteomic analysis by mass spectrometry. In this protocol, BaP is used as an example for treatment, but the process can be applied for other treatments.

Materials:

10 mM Benzo-a-pyrene (BaP) (Sigma, B1760) stock solution in DMSO

100 mm tissue culture plates (VWR, 430167)

Complete media for HepG2 {DMEM (VWR, 10-013-CV), 10% FBS (Gibco, 16000-044), 1% Pen Strep (Gibco, 15140-122)}

DMSO (Sigma, D8418)

On the day of cell lysis

Ice in ice tray

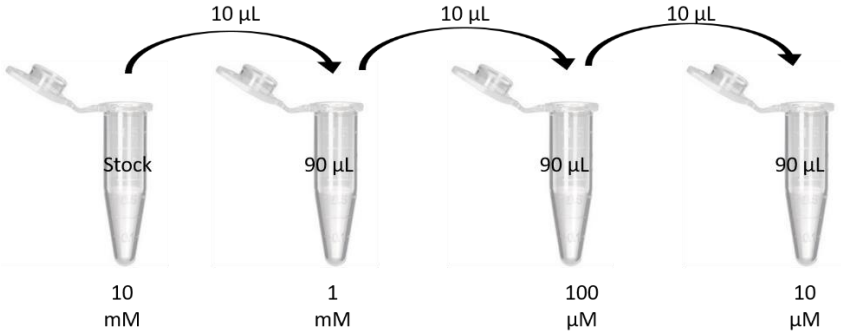
Cold 8M Urea (Sigma, U5128); make fresh

Cold 1x DPBS (Corning, 20-031-CV)

1.7 mL microcentrifuge tubes (VWR, 87003-296)

Preparing Benzo-a-pyrene solutions:

1. Make desirable concentrations of Benzo-a-pyrene by doing serial dilutions. Briefly, add 10 µL of 10 mM stock to 90 µL DMSO to make 1 mM solution. To make 100 µM solution, add 10 µL of 1 mM solution to 90 µL DMSO.



Workflow:

1. Plate 2 million cells per condition in 100 mm dishes in total media volume of 10 mL.
2. Add 10 µL of appropriate BaP solution to cells. For example, for 100 nM treatment, add 10 µL of 100 µM BaP solution to cells. Make sure to have appropriate controls i.e. a plate with DMSO treatment only (10 µL of DMSO to cells).
3. Swirl the plate to mix BaP and cells homogeneously.
4. Place the plates in 37 C incubator, 5% CO2 for desired amount of time.
5. Following the treatment period, prepare the materials for cell lysis:
    a. Make fresh 8M Urea, and put it on ice. Urea can precipitate out of solution at cold temperatures, so make sure to vortex it every 5 mins.
    b. Put the PBS on ice.
    c. Label 1.7 mL tubes for sample collection and place them on ice.
6. Place the plates on ice and aspirate off the media.
   Notes: Some of the post-translational modifications such as phosphorylation are sensitive to temperature fluctuations. Therefore, it is critical to keep the plates on ice throughout the following steps, and work quickly through the steps.
7. Wash the cells with 10 mL ice-cold PBS.
    a. Pour on
    b. Swirl the plate
    c. Aspirate of the PBS
8. Add 1 mL of ice-cold 8M Urea to the plate
    a. Try to cover the entire plate with 8M Urea. Swirl the plate if needed.
9. Use a cell scraper to lift the cells off the plate into the solution.
    a. Scrape in all four directions while holding the dish at a slant in order to remove all of the cells and collect them at one end.
10. Pipette the solution into labeled 1.7 mL microcentrifuge tube, and store it in -80 C until you process the samples for proteomic analysis.