Cell sample preparation for mass spectrometry analysis

Cell harvesting

Adherent cells. This protocol is for a plate that was seeded with $1x10^6$ cells in 100-mm Petri dish before treatment. Biological triplicates (as a minimum) are recommended. The protocol is followed for each plate from start to finish (i.e., plates are not processed in parallel). After the desired time for growth and/or drug treatment, remove and discard the culture medium. Briefly rinse the cell layer with PBS to remove traces of medium. Add 1 ml of trypsin-EDTA solution to the dish and examine cells using an inverted microscope to verify that the cell layer has been dispersed (usually within 2 – 5 min). *Note:* To avoid clumping, do not agitate the cells by hitting or shaking the plate while waiting for the cells to detach (For some cells it may be necessary to keep 37 °C to enhance detachment). Add 6 – 8 ml of complete growth medium and aspirate cells by gently pipetting up and down. Transfer cells and media to a 15-ml Falcon tube and centrifuge at 1500 rpm for 2 min. Remove and discard the supernatant and add 1 ml PBS to the cell pellet. Disperse the cell pellet by gently pipetting up and down two to three times using a 1-ml pipette. Transfer the cells and PBS to a 1.5-ml polypropylene tube with a cap. Centrifuge the cells at 2000 rpm for 2 min. Remove and discard the PBS supernatant.

Cells grown in suspension. No trypsin treatment necessary. Centrifuge cells in 15-ml Falcon tube as described above, remove and discard the supernatant and add 1 ml PBS to the cell pellet. Disperse the cell pellet by gently pipetting up and down two to three times using a 1-ml pipette. Transfer the cells and PBS to a 1.5-ml polypropylene tube with a cap. Centrifuge the cells at 2000 rpm for 2 min. Remove and discard the PBS supernatant.

Freezing

Snap-freeze the tube with the cell pellet in liquid nitrogen and store at -80 °C until analysis.

Submission to MS core

When directed by Sam or Dana, bring the tubes with the frozen cell pellets on dry ice to the designated location.

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