Harvesting and CH_3CN quenching of adhered cells
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ADHERENT CELLS

Note: This procedure follows [Fan_Cell_Tracer_Ex]. Step 6 from that SOP is detailed here.

PREPARATION

1. Cells: Cell density in the plate should be about 80-95% confluence in a 10 cm cell culture plate.
2. Cold CH_3CN: Cool down a bottle of 100% CH_3CN (Optima or HPLC grade) in -20°C freezer overnight before extraction.
3. Ice tray, put following items in the tray
    a. Cold PBS (non-sterile), ultrapure H_2O (MilliQ), and cold CH_3CN
    b. A new 15 ml screw-cap conical tube (Sarstedt, 62.554.002)
    c. A cell lifter (Fisher, 11577692).There is no need to be sterile, so a used but clean one is fine)
    d. The plate(s) with cells

PROCEDURE (All operations should be performed on ice to reduce metabolite degradation. Fill a plastic bag with ice to provide a clear surface. This helps to prevent ice or water from jumping into the plate.

1. Medium removal and Wash (use a vacuum line to suck up cell culture medium and other liquids)
    a. Transfer media into a 15 ml conical tube, centrifuge at 3,500xg for 20 min, 4°C (can be done along with the cell extract below). Remove supernatant and aliquot 100 µl in a 2 ml screw top tube (USA Scientific 1420-8700) for metabolite extraction and freeze remaining media at -80°C for exosomal isolation.
    b. Wash the cells by adding 5 ml of cold non-sterile PBS buffer onto the side of the plate, and gently rotate the plate to wash all surface of the plate. Remove PBS by vacuum-suction. Repeat (2) twice for a total of (3) three PBS washes.
    c. After the 3rd PBS wash add 5 ml MilliQ H_2O, swirl and aspirate off as much water to minimize salt contribution to the extract: DO NOT ALLOW THE CELLS TO SIT IN H_2O LONGER THAN 30 SECONDS!
        i. Let the plate sit tilted on ice to allow as much water to collect at the bottom while aspirating. Keep aspirating liquid in the bottom corner without touching the cells.
2. Quenching and collection
    a. Add 1 ml of cold CH_3CN to cover the whole plate and let stand for ≥ 5 min (can be put at -20C, helps with the cell lysis)
    b. Add 0.55 ml nanopure water + 0.2 ml 0.2 mM Tris-HCl pH 8 as an internal standard for NMR; this is optional) to the plate.

        Note: Prepare a “mastermix” to make sure every sample gets the same concentration of Tris (add water/Tris just before scraping the cells: water will

facilitate vigorous scraping with cell lifter and prevent plastic from coming off the plate in CH_3CN)

c. Scrape cells, and collect cells into a 15 ml polypropylene centrifuge tube (see Fig. 4)
    i. Scrape the surface of the plate using the cell lifter. Do not forget to scrape the edge of the plate.
    ii. Let the plate sit tilted on ice, and use the lifter to push all cells and CH_3CN/H_2O into the bottom corner.
    iii. Collect the cells and the CH_3CN/H2O into the 15 ml tube using a transfer pipette.
d. Repeat step (a) to (c) to collect cells again except that 0.75 ml nanopure water (no Tris) is used.

    Note: The final CH_3CN to water ratio is 2:1.5 (v/v)
e. Combine both collections into the 15 ml tube.

    Note: This is a break point; as soon as the chloroform is added the procedure has to be completed. Chloroform will eventually dissolve the plastic of the 15 mL tube, but it is fine for short-term use.