Harvesting and CH₃CN quenching of adhered cells, suspended cells; CH₃CN quenching of tissue by Teresa Fan, University of Kentucky

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 CH₃CN: Cool down a bottle of 100% CH₃CN (Optima or HPLC grade) in 20°C freezer overnight before extraction.
- 3. Ice tray, put following items in the tray
 - a. Cold non-sterile PBS and cold CH₃CN
 - 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 μ l 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. Add 5 ml of cold non-sterile PBS buffer onto the plate, and gently rotate the plate to wash all surface of the plate. Remove PBS by vacuumsuction.
 - c. Repeat step (b) twice and remove as much PBS as possible after the 3rd wash to minimize salt contribution to the extract:
 - i. Let the plate sit tilted on ice for about 1 min. 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 \geq 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 CH₃CN)

- 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 CH₃CN/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 CH₃CN 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.

SUSPENDED CELLS

- 1. Centrifuge cells @ 281xg, 4°C for 5 min to pellet the cells
- 2. Collect the medium supernantant, save 0.1 ml in a 1.5 ml microfuge tube and also save 1.5 ml in a separate 2 ml screw cap tube for safekeeping at -80C
- 3. Resuspend the cell pellet in 10 ml ice-cold PBS and centrifuge at 281xg, 4°C, 5 min
- 4. Remove as much PBS wash as possible
- 5. Resuspend the cell pellet in 0.5 ml ice cold PBS and transfer the suspension to a pretared 1.5 ml microfuge tube (record the tare wt); wash the cell tube with another 0.5 ml ice cold PBS and pool with the 1st 0.5 ml suspension
- Centrifuge the pooled cell suspension at 1700xg/4°C/5 min to hard pellet the cells
- 7. Remove as much as possible PBS (this is an important step to minimize salt and to get more accurate wet wt. You can use a gel loading tip at the end of a vacuum line to suck up the buffer or use an ultra-fine pipet tip to remove PBS. The former method is preferred)
- 8. Immediately after removing PBS, wipe out any moisture on tube exterior, weigh the tube, and flash-freeze in liq N2.

TISSUE QUENCHING

- Add 2 ml of cold CH₃CN to cover the frozen tissue power (pulverized in liq. N₂ TO fine powder) in 15 ml polypropylene conical centrifuge tube (Sarstedt) and let the powder thaw on ice.
- Add 0.75 ml nanopure water + Tris-HCl (pre-mixture of 0.55 ml nanopure water + 0.2 ml 0.2 mM Tris-HCl (pH 8)) (as internal standard; this is optional) to the tube.

3. Add 0.75 ml nanopure water (without Tris) to the tube and vortex rigorously to maximize extraction.

Note : total amount of water should be 1.50 ml and the ratio of CH_3CN to water should be 2 : 1.5.