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Stable Isotope Tracer in Adhered Cells Experimental Protocol

1. Grow cells in 10 cm plates to about 70% confluent in an appropriate medium and conditions

2. One day prior to or on the day of the treatment, prepare stable isotope tracer medium. For example, uniformly ^{13}C-glucose ($^{13}C_6$-glucose) is added to RPMI or DMEM medium without glucose [a] at 0.2 to 0.45%, depending on cell's glucose demand [b]. Also added is 10% dialyzed (10 kDa molecular weight cutoff) [c] FBS, 1X streptomycin-penicillin, 2 to 4 mM glutamine, and any other components needed. Incubate all media at 37°C, 5% CO_2 to pre-equilibrate temperature and pH.

   [a] If another tracer such as $^{13}C_5$,$^{15}N_2$-Gln is desired, use medium without both glucose and Gln and supplement the medium with the corresponding labeled tracers.
   [b] It is important to have excess tracers to avoid depletion at the end of the treatment
   [c] If cells do not grow well in dialyzed FBS, use regular FBS instead.

3. Remove old medium thoroughly from plates using vacuum suction but without drying the plate. Rinse the plate with 5 ml medium without any supplement and vacuum suction to remove residual lactate from cells and old medium components. Record the weight of each blank plate using a sterilized three-place balance placed in the biosafety hood. (g tare plate)

4. Transfer 8 to 10 ml of pre-warmed freshly prepared tracer medium to each blank plate, remove 0.2 ml aliquot from plate and store in a screw-cap 1.5 ml microfuge tube. Record the weight of blank plate+medium. (g tare plate+med @ 0h) Transfer 0.1 ml of the medium aliquot into a snap-cap 1.5 ml microfuge tube and record the weight of the transferred medium using a 4-place balance. (g 0.1ml med/0h) Take a snap shot of each plate (at 100X magnification) to record cell distribution and morphology

   Note: This process is best done with two people with no more than 8 plates at a time to avoid leaving cells out of the incubator too long. The medium weight is important to correct for medium loss due to evaporation.

5. Incubate cells in tracer medium at 37°C, 5% CO_2 and record the start time. If time course of the medium is desired, record the weight of the plate before removing 0.2 ml aliquot at different time intervals (e.g. 3, 6, 12, and 24 h), and record the weight of the plate again after medium removal. (g tare plate+med @n h - weight of plate at time point, g 0.1ml med/n h – weight of sample)

   Note: For initial survey, 24 h of tracer incubation or duration comparable to the doubling time of the cell is good to adopt.

6. At the end of the tracer treatment, record the weight of the plate (g tare plate+med @n h - weight of plate at time point), and take another snap shot of each plate (at 100X magnification) to record cell distribution and morphology. Place plate on ice as soon as possible, transfer the medium into a 15 ml centrifuge tube and centrifuge at 3500xg or higher speed for 20 min at 4°C to remove cell debris. Aliquot 0.1 ml medium supernatant into a snap-cap 1.5 ml microfuge tube and record the weight. (g 0.1ml med/n h – weight of sample). Store the rest of the medium in a 7 ml screw-cap vial at -80°C. Immediately after medium removal, wash the plate on ice with ice-cold PBS for 3 times via vacuum suction. Follow the cell quenching and extraction protocol to recover polar, lipid, and protein fractions from plates.

   Note: It is important to minimize the plate processing time and to keep plate cold at all times (e.g. < 10 min) to minimize further metabolic changes.