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Cell Sample Preparation for Metabolic Phenotyping

Disclaimer

The optimal method for cell sample preparation for metabolic phenotyping depends on the cell type. biocrates life science ag offers these guidelines as a suggestion. The methods described here have been shown to work for several cell lines. The cell sample preparation methods suggested here may not be suitable for the specific cell type used in your metabolomics project.

General considerations

- The number of cells necessary depends on the assay and the cell type. We usually recommend providing 1-3 x 10^6 cells per sample, however for small cells or to ensure detection of low concentrated metabolites, higher cell numbers may be necessary.
- Ensure that cells on all plates have similar density, are in exponential growth rate, and have the same proliferation rate (unless opposed by the experimental set-up).
- We suggest providing at least 10 biological replicates per condition to even out natural fluctuations. For a statistically meaningful evaluation, there should be at least 30 samples per group.
- Label the sample storage vials before starting the cell sample preparation. Please ensure the labels are waterproof and resistant to cold storage conditions.
- Do not harvest the cells using trypsin, accutase, or other enzymes. The enzymatic treatment affects the metabolome and thus has impact on the measured metabolite concentrations. Stick to the preparation methods below.
- Please keep all processing procedures and times standardized and use identical storage tubes in a single study to ensure comparability.
- If one of the methods that involve cell counting is employed, count after the samples have been frozen or get a second person for the cell counting to avoid delays.
- If culture medium samples are to be analyzed, consider adding a sample of fresh medium to obtain reference concentrations. The volume of culture medium necessary for analysis depends on the assay and is included in the offered quote. All cell culture medium samples are to be frozen as soon as they are obtained.
- Transport the frozen samples to Biocrates on dry ice according to shipment instructions.
- Biocrates prefers sample shipments in 1.5 mL or 2 mL tubes.

Cell pelleting

Cell pelleting is the method of choice for all non-adherent cells (growing in suspension) as well as non-adherent 3D cultures such as spheroids.
The appropriate amount of suspension culture is simply transferred to a tube suitable for centrifugation. Before centrifugation, a small aliquot should be taken for cell counting. The suspension is centrifuged at room temperature at a speed that will not destroy the cells (dependent on the cell type). After formation of a cell pellet, the supernatant is removed. The supernatant may be preserved for a separate analysis of the culture medium if desired. If it is necessary to transfer the cells to a smaller tube for freezing and shipment, the cell pellet may be carefully resuspended in 0.9% NaCl (saline), transferred to a smaller tube and pelleted by centrifugation as before. After removal of the supernatant, the cell pellet is to be frozen quickly (liquid nitrogen is recommended) and stored at or below -80 °C until shipment to biocrates.

Cell scraping

Cell scraping is suitable for all adherent cells that detach easily from the plate or equivalent (such as HEK293A cells) as well as organoid cultures and adherent cells that do survive the cell scraping process.
This method is derived from a protocol used at the University for Heidelberg, Germany. This procedure is stressful for the cells and this may change the metabolome, thus it is important to cool the cells and work quickly. The cell medium is removed, an aliquot may be preserved for a separate analysis of the culture medium if desired. The cells are washed with cooled 0.9% NaCl solution (saline, 4°C). After removal, a small volume (1.5 mL recommended for a 9 cm plate) of pre-cooled 0.9% NaCl solution is added to the plate. The cells are then quickly detached with a cell scraper. The cell suspension is to be transferred to a 1.5 mL or 2 mL tube suitable for centrifugation. Before centrifugation, a small aliquot should be taken for cell counting. Be aware that the cell suspension should be homogeneous to increase the counting accuracy. The sample is centrifuged at 4°C at a speed that will not destroy the cells (dependent on the cell type). After formation of a cell pellet, the supernatant is removed. The cell pellet is to be frozen quickly (liquid nitrogen is recommended) and stored at or below -80 °C until shipment to biocrates.

Cell extraction

Cell extraction is suitable for all adherent cells.
This method was adapted from Brunelli and Caiola et al. (doi: 10.1038/srep28398). The mode of action does not allow cell counting after harvesting, so the cell number must be estimated from the number of seeded cells. The procedure is stressful for the cells and this may change the metabolome, thus it is important to work quickly. The cell medium is removed; an aliquot may be preserved for a separate analysis of the culture medium if desired. The cells are quickly rinsed with pre-cooled 0.9% NaCl solution (saline, 4°C), and metabolism is quenched by adding liquid nitrogen to the plate. The amount of liquid nitrogen depends on the plate or well size, use about 15 mL for a Ø 15 cm dish. The plates are stored at -80 °C and extracted within 7 days. Alternatively, the extraction can be performed directly without freezing the cells.

Extraction has to be performed on ice. The lowest possible volume of ice-cold methanol (or methanolic extraction buffer (90% methanol in H_2O)) is added to the plate, we recommend 25 µL per 10^6 cells. The cells are then rapidly detached with a cell scraper. Extracts are swiftly transferred to 1.5 mL micro-centrifuge tubes and frozen (liquid nitrogen is recommended). At this point, you can either stop and leave the rest to biocrates (costs for cell extraction will apply) or continue the cell extraction as follows. To break down the cell contents, thaw frozen cell pellets on ice, sonicate in an ice bath for 3min, and snap-freeze in liquid nitrogen for 30 sec. Thaw, sonicate and freeze a second time, then thaw and sonicate a third time. Centrifuge samples for 5 min at 2°C at full speed (at least 18000 rpm). Transfer the supernatant to a new labeled tube and freeze in liquid nitrogen instantly. Discard the old tube with the pellet. The tubes are stored at or below -80 °C until shipment to biocrates.

Sample shipment

- Please inform the analytical laboratory about the sample shipment 2 to 3 days before the actual shipment.
- Please provide a tracking number.
- Please provide an electronic sample list (use Excel template provided by biocrates).
- Ship the samples on sufficient dry ice (minimum 10 kg for Europe, 20 kg overseas, thick-walled styrofoam container); the samples should be in labeled boxes protected by a plastic bag.
- The analytical lab will be able to receive samples on working days (8 a.m. to 5 p.m.).