Digestion of cultured cells

Sample Preparation

For *adherent cultures*, cells or bacteria are rinsed twice with an isotonic solution prior to detaching. This will remove most of the external trace elemental content. Typical detaching solutions are TrypLE (Thermo Fisher Scientific) but this will depend the cell type. Cells or bacteria are harvested directly into 15ml centrifuge tubes (metal free, VWR, catalog number 89049-170) containing an equal amount of medium. After removing an aliquot for cell count or protein determination the cells are spun down and as much medium as possible is removed. For cell counts, trypan blue can be used to estimate the number of live/dead cells (> 90% live is best).

Cells or bacteria *grown in suspension* are harvested as described above with the exception of the detaching step. A cell count is taken before the cultures are spun down. The cell pellet is rinsed with an isotonic solution to remove any growth medium and the cells are spun down again.

Ideally, we would want 10^6 cells per sample but we can live with 10^5. Cell pellets (medium removed) should be stored at -80˚C until digested or shipped

The following work will be performed in the Elemental Analysis Core at OHSU unless you agreed to do this in your lab.

Digestion

100 µl of concentrated HNO_3 (trace metal grade, Fisher) is added to each cell pellet (containing approximately 10^6 cells). The tubes are loosely capped, and the samples are heated for 45 min at 90˚C in a heating block. After digestion, 900 µl of 1% HNO_3 (prepared from trace metal grade HNO_3, Fisher) is added to each sample.

Controls

The following digestion/background controls (each in triplicate):

- *NIST bovine liver standard (SRM 1577c). ~10 mg (weighed on a precision balance (+/- 0.1 mg)) is added to 200 µl of 50% HNO_3 (prepared from trace metal grade HNO_3, Fisher) and heated for 45 min at 90˚C in a loosely capped 15ml centrifuge tube (metal free VWR, 89049-170).*
- *Calibration standard. 2 µl of calibration standard #2 (CEM2, VHG-SM70B-100) is added to 200 µl of 50% HNO_3 and heated for 45 min at 90˚C in a loosely capped 15ml centrifuge tube (VWR, 89049-170).*
- *Blank. 200 µl of 50% HNO_3 (prepared from trace metal grade HNO_3, Fisher) is added to an empty tube and heated for 45 min at 90˚C in a loosely 15ml centrifuge tube (VWR, 89049-170).*

Shipment

Please ship all samples on DRY ICE, NOT AT room temperature!

Ship to:
Martina Ralle
Department of Molecular and Medical Genetics
Oregon Health and Science University
School of Medicine
3181 SW Sam Jackson Park Rd
Portland, OR 97239