

ICP-MS Metal Analysis Protocols

Sample preparation (cell lysis):

When cell pellets are to be normalized to protein concentration, they are first lysed and part of the sample is used for the BCA assay and the remainder is available for ICP-MS analysis. The cell pellet is resuspended in at least four volumes of pure, distilled water (minimum volume 80 uL). The cell pellet is lysed on ice, using 5 pulses with a cell disruptor/sonicator microtip.

Sample preparation (tissue homogenization):

Between 20 and 200 mg of weighed tissue is placed in a Ceramic Bead Tube on ice (with 1.4 mm ceramic beads, MolBio Laboratories Catalog number 13113-50, available from Qiagen). Four volumes of cold, pure, distilled water is added (assuming 1 mg = 1 uL). The tissue is homogenized with vigorous shaking using an Omni beadruptor bead mill. The homogenate is removed from the bead tube, and stored frozen.

Protein concentration determination for normalization (BCA Protein Assay):

A Pierce BCA Protein Assay Kit (Thermo Scientific REF 23225) is used to determine protein concentration in cell lysates and homogenates. BSA standards for the assay are created ranging from 0.1 to 0.8 ug/uL. Serial two-fold dilutions (in 0.1 N NaOH) of lysates or homogenates are generated in a dilution plate. Optimal dilutions can start from 1:5 for cell lysates (can be lower) and up to 1:320 for homogenates (can be higher). For the assay, 10 uL of diluted lysate/homogenate or standard is added to each well of a 96 well plate containing 200 uL assay mixture, and the BCA kit protocol is followed. We run a range of dilutions for each sample, and use readings (at 562 nm) that fall between OD 0.15 and 0.36 for calculating protein concentration. Each sample dilution is run in duplicate.

Sample preparation (digestion):

For each ICP-MS replicate, 30 to 50 uL of homogenate or cell lysate is digested using a 5:1 mixture of nitric acid (OPTIMA grade, 70%, Fisher Scientific) and ultrapure hydrogen peroxide (ULTREX II, 30%, Fisher Scientific). 30-50 uL lysate/homogenate, 500 uL of nitric acid and 100 uL hydrogen peroxide are added to 2 mL polypropylene tubes (VWR 10011-742, Axygen REF MCT-200-C clear, homo-polymer, boil-proof). This mixture is allowed to digest overnight at room temperature, heated at 95°C just until dry, and resuspended overnight in 1 mL of 2% nitric acid for analysis. The mixture is pipetted up and down the following day to ensure resuspension, and 0.8 mL more 2% nitric acid is added along with 0.2 mL of 10X internal standard (prepared in 2% nitric acid).

ICP-MS run (simplified description):

An Agilent 7900 ICP-MS is operated in helium (He) collision cell gas mode for all measurements. Elements are measured at the following isotopes (only selected elements shown): ^{56}Fe , ^{55}Mn , ^{63}Cu , ^{66}Zn , ^{72}Ge , ^{45}Sc , ^{59}Co , ^{60}Ni & ^{85}Rb . Calibration standards and samples are prepared in an acid matrix of 2% OPTIMA Grade Nitric Acid. Solutions of Agilent Multi-element Calibration Standard 2A are prepared to obtain an eight-point calibration curve. Agilent Environmental Calibration Standard is typically used as an independent measure. Agilent Germanium (or Scandium) Standard(s) are added to calibration standards, blanks and samples, and are used to correct for potential sample matrix and/or nebulization effects.

General Fe analysis description for our instrument:

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an instrumental technique used to quantitate most elements of the Periodic Table from sub-ppt to percent levels in solution. The mass spectrometer isolates and counts individual isotopes of analytes of interest for quantitation based upon their mass to charge (m/z) ratio. An Agilent 7900 ICP-MS was operated in helium (He) collision cell gas mode for all measurements. Helium cell gas mode is used to remove polyatomic interferences, where ever they occur, on analytes of interest by a mechanism known as Kinetic Energy Discrimination. In this work, Fe is measured at its most abundant isotope of ^{56}Fe and He cell gas effectively removes both $^{40}\text{Ar}^{16}\text{O}$ and $^{40}\text{Ca}^{16}\text{O}$ interferences on it.

This instrument is equipped with a MicroMist glass concentric nebulizer, quartz spraychamber (cooled to 2 °C), quartz torch with 2.5 mm internal diameter injector and standard Nickel interface cones. An Agilent SPS4 autosampler is used to deliver the samples from a 96 position deep-well polypropylene microplate. Agilent ICP-MS MassHunter 4.4 software allows autotune of all lens within the instrument. Run parameters were as follows: RF Power 1600 W, Sample depth 8.0 mm, carrier gas 1.05 L/min, Sample flow rate: 0.2 mL/min, Cell gas flow rate 5 mL/min.

Calibration standards and samples are prepared in an acid matrix of 2% OPTIMA Grade Nitric Acid. Calibration standards for Fe are prepared using Agilent Multi-element Calibration Standard 2A to obtain an eight-point calibration curve. Agilent Environmental Calibration Standard is used as an independent measure. Agilent Germanium (or Scandium) Standard(s) are added to standards, blanks and samples and are used as internal standards to correct for potential sample matrix and/or nebulization effects.